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Bulletin of the Museum of Comparative Zoölogy

AT HARVARD COLLEGE.

VOL. XXVII. No. 1.

SPERMATOGENESIS OF CALOPTENUS FEMUR-RUBRUM AND CICADA TIBICEN.

BY E. V. WILCOX.

WITH FIVE PLATES.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
MAY, 1895.

No. 1. — *Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen.*[1] By E. V. Wilcox.

The following observations were made on the testes of Cicada and Caloptenus. Only three male Cicadæ were at my disposal, but of Caloptenus I examined more than twenty individuals. The Cicadæ were killed immediately after leaving the pupal case, and had been preserved a number of years. The Calopteni were taken in August and September, 1893.

The testes of Cicada were killed in Müller's fluid; those of Caloptenus either in hot water, in hot corrosive sublimate, in cold corrosive sublimate, or in chrom-osmic-acetic mixture. Some of the testicular follicles of Cicada were stained in Grenacher's alcoholic borax carmine, others according to Bizzozero's modification of Gram's method. The follicles were stained *in toto* in safranin (50% alcohol) 24 hours, sectioned, stained 3 minutes in gentian-violet, washed 5 minutes in a solution of potassic iodide, then treated alternately with alcohol and chromic acid (0.1%). But better results were obtained by double staining with safranin and victoria-green. Crystals of the latter were dissolved in absolute alcohol, or in clove oil. The sections were first stained in safranin (10–15 minutes), the excess of stain being quickly washed off in 90% alcohol, and then in a very strong solution of victoria-green in absolute alcohol for 1 to 2 minutes. Staining and dehydrating were thus accomplished at the same time. Excess of green was washed out with absolute alcohol. Sections were cleared in clove oil. When a clove-oil solution of the green was used, the sections were dehydrated before staining in the green. The method with the absolute-alcohol solution gave the better results, and was more easily managed.

The Caloptenus material was all stained on the slide. The methods used were either safranin and victoria-green, as just described, Henneguy's ('91) potassic permanganate and safranin, or Heidenhain's ('92) iron-hæmatoxylin. The method with safranin and victoria-green gave good results. Cytoplasm and achromatic nuclear parts were stained green, the chromosomes, nucleolus, and centrosomes red. If the green be allowed to act too long, it will replace the safranin entirely. This (safranin and green) method was the only one by which the archoplasm was made distinct. The granular cytoplasm was stained green, and in the pale green clear areas of the archoplasm were to be seen the red centrosomes. In *some* stages the chromosomes were stained green, indicating that a chemical change takes place in the chromatic substance. But even in such cases the nucleolus was bright red.

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. XLVII.

In using Henneguy's method, the sections were put into permanganate of potash for 5 minutes, and then stained 3–20 minutes in Zwaardemaker's safranin. The mordant gives an iron-rust color to the sections, and the safranin must not be too much washed out, or the sharpness of outlines will be lost. It is best to wash out the mordant very thoroughly before using the stain, for the potassic permanganate makes a precipitate with the safranin which renders the sections so muddy as to be nearly useless. By this method the chromosomes and nucleoli are stained bright red, the individual chromosomes being sharply outlined. In the metamorphosis of the spermatid, the six spheroidal chromatic elements are often easily distinguished, although closely massed together. The chromatic crescent of the spermatid is very well defined; but the small body in the neck of the spermatozoön, so conspicuous after treatment by Heidenhain's method, is hardly to be seen when this method is used. Centrosomes were rarely stained; achromatic fibres of ring stages were faintly stained; the nucleus often appeared as a clear lenticular space, in which were the red chromosomes.

The best results were obtained by use of Heidenhain's method. The "black" process proved more serviceable than the "blue." The only mordant used was double sulphate of iron and ammonia, $NH_4Fe_2(SO_4)_4$. A 2% aqueous solution was used as mordant, and a 4% aqueous solution as decolorizer. To produce the "blue" stain, the sections were placed in the mordant $\frac{1}{2}$–1 hour, and after washing in water were stained in the hæmatoxylin (0.5% pure hæmatoxylin in H_2O) 1–2 hours; finally, they were washed again in water. Sometimes it is necessary to decolorize a short time, say 20 minutes, in 4% $NH_4Fe_2(SO_4)_4$. The "black" stain was obtained by leaving the sections in the mordant 2 hours before washing in water, staining 10–12 hours in the hæmatoxylin, and decolorizing 2–8 hours, finally washing as before. For either process the sections should be very thin. They must be firmly affixed to the slide; for the washing is best done by a stream of tap-water allowed to run over the slide. Three washings are necessary, each of which should be thorough: (1) after use of the mordant,

(2) after staining, (3) after decolorizing. Simple immersion in water does not do as well. The mordant and stain will form a precipitate, just as in Henneguy's method, and if the first washing be neglected, it is next to impossible to remove the precipitate by subsequent washings. One to five minutes in a stream of water is enough for each washing. The sections will become quite opaque immediately after immersion in the decolorizer, but in this the opacity is slowly removed. The decolorization is hastened by washing the sections in water at intervals during the process of decolorizing. This is necessary, also, in order to see how far the decolorizing has progressed. The process can thus be stopped at the desired stage. The proper decolorization is the most difficult part of this method.

By the "blue" process, so far as my experience goes, the cytoplasm stains gray, the centrosomes do not stain at all, the spindle and linin fibres very faintly, the chromosomes dark blue. By the "black" process the cytoplasm takes a dark-gray color, and both centrosomes and chromosomes are made black, while spindle fibres and linin fibres become very distinct. The nucleoli are colored nearly black by either process.

CICADA TIBICEN.

The testes of Cicada tibicen are paired, and each consists of a large number of ellipsoidal follicles, which are closely packed together. The follicles of each side of the body open into a vas deferens, which soon joins its fellow of the opposite side. Figure 14 (Plate I.) gives an idea of the spatial relationship to one another of different spermatogenetic stages. It represents a very nearly longitudinal section of a follicle of Cicada. At *a* are spermatogonia; at *d*, spermatids in various stages of metamorphosis.

The Cicada material at my command did not show the division stages, but it gave a very reliable series of preparations on certain other stages.

The spermatogonia lie at the blind end of the follicle. They occupy in my preparations only the single end-compartment (Fig. 14, *a*). Their size is less than that of the spermatocytes, and they are further distinguished from them by the fact that they have only 12 chromatic rods, whereas the spermatocytes have each 24 spherical chromosomes. One or often two nucleoli are to be seen.

The spermatocytes occupy usually two compartments next to that of the spermatogonia. The chromatic substance consists of about 24 spheroidal bodies. In Figure 14 the compartment *b* contains spermatocytes of the first order; *c*, spermatocytes of the second order and spermatids just after the last division. (Compare Fig. 23 and Explanation of Figures.) The cells of *b* each contain one or two bodies which I consider nucleoli, since they react to the stains quite differently from the chromosomes. Figures 53–59 (Plate II.) represent cells quite commonly met with among the spermatocytes; they are numbered in the order in which I think they succeed one another. In a single compartment may be found spermatocytes in several different conditions; the earliest seems to be that in which the nucleolus lies in the centre of the nucleus with the chromatic spherules arranged radially about it (Fig. 53). The nucleolus then moves to the periphery of the nucleus, and appears meantime to have divided into two portions (Figs. 55, 56), one of which passes into the cytoplasm, while the other remains in the nucleus (Figs. 58, 59); later, both parts appear outside the nucleus and on diametrically opposite sides of it.

Hertwig ('90) has noticed the disappearance of the nucleoli in the spermatocytes of Ascaris megalocephala just before the appearance of the centrosomes. Brauer ('93) figures the centrosomes as arising singly in each nucleus and dividing either inside (univalens) or outside (bivalens) the nucleus, according to the type. But Brauer saw nucleoli in the same nucleus with the centrosomes and differing from them in stainability. Born ('94) maintains that the nucleoli have nothing to do with either reproduction or cell division. He says: "Die Nucleolen stehen in Beziehung zum individuellen Zellleben, nicht zur Fortpflanzung, denn beim Beginn der Mitose verschwinden sie um nach Beendigung derselben —im Ruhezustand des Kerns — wieder aufzutreten."

Thus the nucleoli have been supposed to give rise to the centrosomes, to be modified chromatin, — a stage in the evolution of a chromosome, — to be excretory organs of the nucleus (Häcker, '93), or to serve some unknown function in the economy of the cell (Born, '94). The nucleoli are found by Born to be very numerous and large in the germinative vesicle of the egg of Triton during the time when the chromatin is inconspicuous; but they disappear entirely before the formation of the first polar globule.

Since there is such disagreement about the origin, function, and fate of the nucleoli, it is probable that different structures have been called nucleoli by different authors. The several bodies in Cicada seen in and near the nucleus in Figures 50 and 53–61 (Plate II.) — in Figures 53, 54, as a single body, in Figures 55, 56, as two bodies, in

Figures 57–59 as two bodies, one of which is outside the nucleus, and in Figures 50, 61, as two bodies, both outside the nucleus — seem to me to give evidence of being stages in the history of one and the same body. My reason for thinking that they are genetically connected is their similarity in size, structure, and reaction to stains.

During the stages shown in Figures 49, 51, 52, there appears to be a chemical change in the constitution of the chromosomes. By the safranin and victoria-green method the chromosomes stain red, though not so deeply as the nucleoli. At later stages the chromosomes assume a green color, while the nucleoli continue to stain red. In still later stages (as Figs. 50, 60, 61) the chromosomes again take the red.

The metamorphosis of the spermatid could be worked out in considerable detail. The chromatin is first arranged around the periphery of the nucleus (Plate I. Figs. 24, 27–30). The individual chromosomes fuse into a thin shell of chromatin, surrounding, in part, the nuclear space. This chromatic shell does not extend over the whole periphery of the nucleus, and yet it is so extensive at the beginning of the metamorphosis that in certain views of the nucleus it has the appearance of a complete sphere.

Figures 15–18 and 24–45 show various stages in the spermatid metamorphosis. Figures 62–77 (Plate II.) present a series of the changes which take place in the head of the spermatid. The stage in which the chromatin (Figs. 66–72) has the form of a crescent is very common, and therefore undoubtedly of considerable duration.

The origin of the extranuclear body (Nebenkörper), which is stained dark green in Figures 20, 27–30, could not be determined. On the anterior end of the nearly mature spermatozoön (Fig. 1 *e*) is to be seen a highly refractive curved tip. Just behind it is a small darkly stained body. The body so conspicuous in the neck of the spermatid of Caloptenus (Plate V. Figs. 196–200) was very rarely seen in the Cicada, probably because the methods used on Cicada would not stain it.

Degenerating cells are very frequent in the testicular follicles of Cicada. So far as my work on Cicada and Caloptenus goes, amitotic division and degeneration affect only the spermatogonia, i. e. if the reproductive cell reaches the spermatocyte stage, it completes its course. The first sign by which I was able to recognize that a spermatogonium is becoming abnormal is due to a chemical change in the nucleus. The chromosomes stain more brightly than in normal cells. The cytoplasm becomes clearer and more homogeneous. Then the chromosomes become irregular in shape, lose their individuality and fuse into a single mass, as in

Plate I. Figs. 7, 19 (see Explanation of Figures), Plate V. Figs. 204, 210, 211). This mass may be slightly vacuolated and may show nucleoli. Some stages of degeneration in the testes of Caloptenus are shown in Plate V. Figs. 202–220. The nucleus may divide amitotically once, twice, or three times (Figs. 206, 212, 214, 215). This may result in ragged granules or strands, or in regular chromatic spheroids (Figs. 205, 207, 220). It is evident from Figures 202–220, since all are drawn to the same magnification, that some degenerating cells increase enormously in size. These degenerating cells are very numerous in Cicada and Caloptenus, especially in the latter.

In Cicada there are frequently seen spermatozoa and various stages in the metamorphosis of spermatids which are four or five times as large as the corresponding normal forms; they may be called giant spermatozoa. Normal spermatids are represented in Plate I. Figs. 2, 3, 4, 9. Stages in the metamorphosis of giant spermatozoa, drawn to the same magnification, are shown in Figures 12, 13, 21, 22. Figures 78–103 (Plate II.) represent giant spermatid cells found accompanying normal cells. Figure 104 shows normal spermatids, the magnification being the same as in Figures 78–103. The striking similarity between the corresponding stages of a normal spermatid and giant forms is very readily seen on comparing Figures 62–77 with Figures 78–86. The first series is much more highly magnified than the second, hence the apparent equality of size.

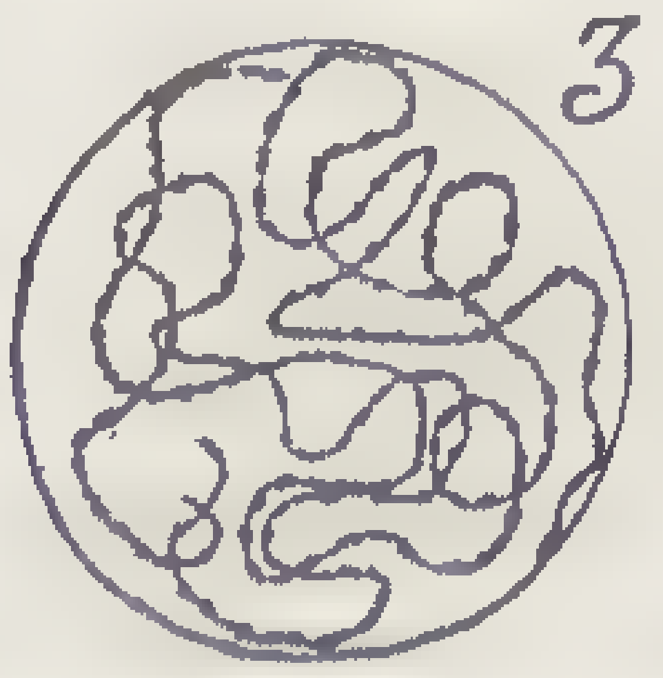
Figures 202, 203 (Plate V.) represent the only examples found in Caloptenus which resemble the giant spermatozoa of Cicada.

What is the meaning of giant spermatozoa? Frenzel ('91, '91^a), Löwit ('91), vom Rath ('90, '91, '93), Verson ('91), Ziegler ('91), Ziegler und vom Rath ('91), Flemming ('89), Geberg ('91), and Meves ('91), have discussed the question of amitotic division, *Lochkerne*, *Ringkerne*, and other degeneration conditions. I believe with vom Rath ('93), that "Alle Zellen welche einmal amitotische Kernteilung erfahren haben, können sich unter keiner Bedingung mehr mitotisch teilen, sie gehen vielmehr einem sicheren Untergang entgegen." I believe that the giant spermatozoa are not functional, that they are excluded from the developmental series and really come to naught. But they arise in Cicada directly from spermatogonia without cell division, by a metamorphosisis of the nucleus, which may or may not be accompanied by amitotic division of the nucleus. In such spermatogonia the nuclei may divide amitotically two or more times (Fig. 8). Then, in the majority of cases, the chromatin breaks up into numerous fragments, which are scattered about the cell irregularly after the nuclear membrane degenerates. Or the fragments may fuse into one mass, which subsequently breaks up. But quite often in Cicada the metamorphoses of these nuclei are rather regular, presenting stages very similar to those which the normal spermatid undergoes. I am not aware that any one hitherto has suggested that the giant spermatozoa arise directly from spermatogonia, and *a priori* it seems, I admit, quite improbable; yet my Cicada preparations point very strongly to this conclusion.

CALOPTENUS FEMUR-RUBRUM.

The testes of Caloptenus consist of tubular follicles, which lie closely packed together, parallel to one another. They are of nearly the same size throughout their length, being slightly larger near the blind end of the tubule, and tapering thence into the collecting duct, which opens into the vas deferens. In my Caloptenus material, taken in August and September, the spermatogonia were confined to a single compartment at the blind end of the tubule. After the spermatogonia the other stages follow in regular succession, a considerable part of the follicle being occupied by the prophases of the first division of the spermatocytes. Then follow regions in which the two successive cell divisions are taking place, then the spermatid metamorphosis, and finally the nearly mature spermatozoa, which with the degenerating cells entirely fill the lumen of the tubule. Figure 108 (Plate III.) represents a longitudinal section of a follicle, in which spermatogonia are shown at *a*, prophases of the first spermatocyte division at *b*, the first division at *c*, spermatids at *d*, immature spermatozoa at *e*, and degenerating cells at *f*. The stages of spermatogonia preparatory to division are seen in Plate III. Figs. 105–107, and Plate IV. Figs. 164–168. Spermatogonium divisions are shown in Plate III. Figs. 119–121, 124, 131, 138, and Plate IV. Figs. 169–171, and a tripolar division at Plate IV. Fig. 189. I could not determine how many divisions the spermatogonia undergo. The chromosomes in the prophases are twelve in number, twenty-four at the equator of the spindle, during metakinesis. The individual chromosomes are rod-shaped or often elongate spindle-shaped. In metakinesis they show ordinarily the well known **V**-shaped figures, and are connected with each other in pairs by means of linin fibres. The centrosomes are usually apparent (Plate III. Figs. 105, 132). Figure 105 shows the centrosome surrounded by a clear protoplasmic area. In most cases a nucleolus is to be seen during the prophases. In Figure 106 there is in the nucleus a body (nucleolus?) which seems to have recently divided.

Before describing the history of the spermatocytes, I will call attention briefly to the figures which illustrate their various conditions. The earliest stage of spermatocytes that I have found is shown in Plate III. Figs. 110, 111. In Figure 111 the cell is not complete, part of the chromatin having been cut away in the previous section. Figure 221 (Plate V.) is of about the same age. Figures 114, 116 (Plate III.), and 184–186 (Plate IV.) give an idea of a slightly older stage. Figures 187, 188 (Plate IV.), and 222 (Plate V.) are still older. In these the chromatic thread is already broken up into segments. Figures 228, 229, 242, 243 (Plate V.), and 175, 176, 178 (Plate IV.), show various stages in the ring and "Vierergruppen" formation. During the first spermatocyte division the chromatic Vierergruppen are arranged at the equator of the spindle, essentially as shown in Figure 175 (Plate IV.). Figures 237–241 (Plate V.) illustrate the first division of the spermatocytes.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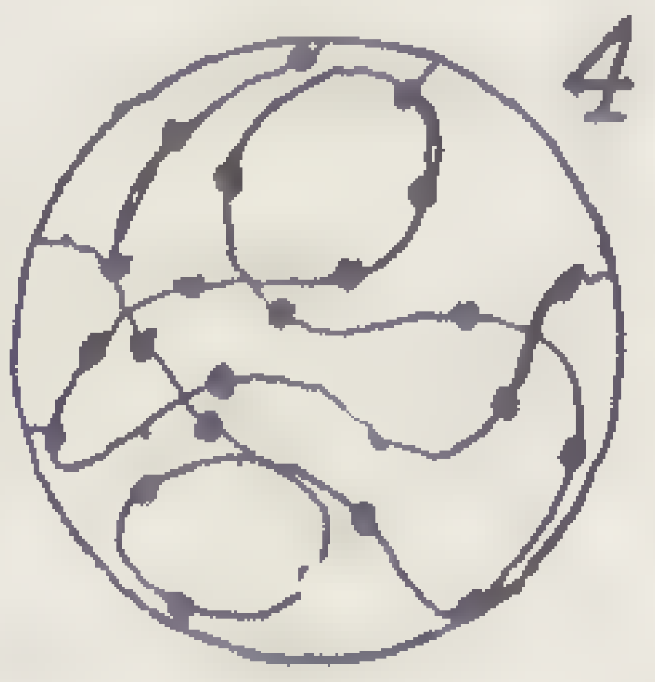
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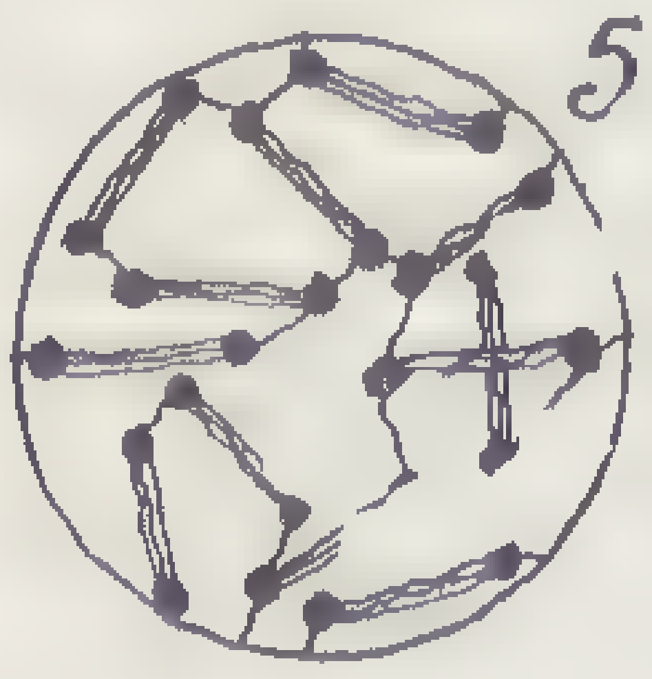
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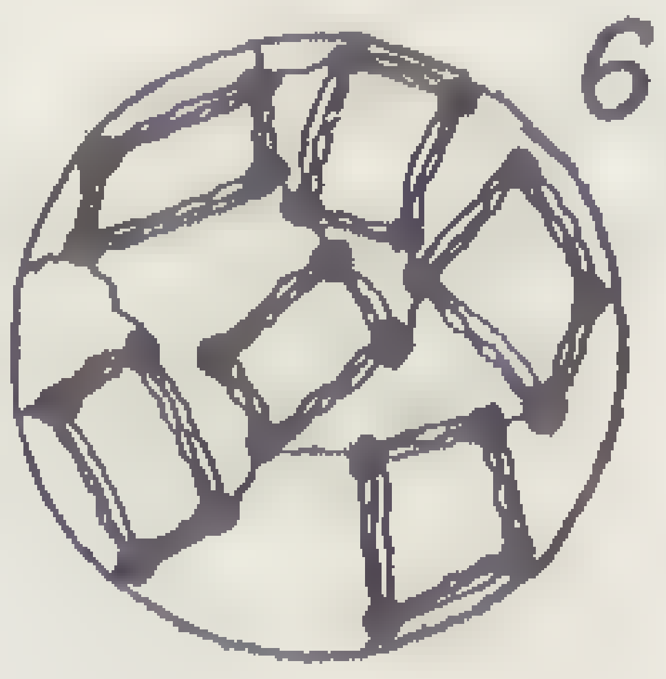
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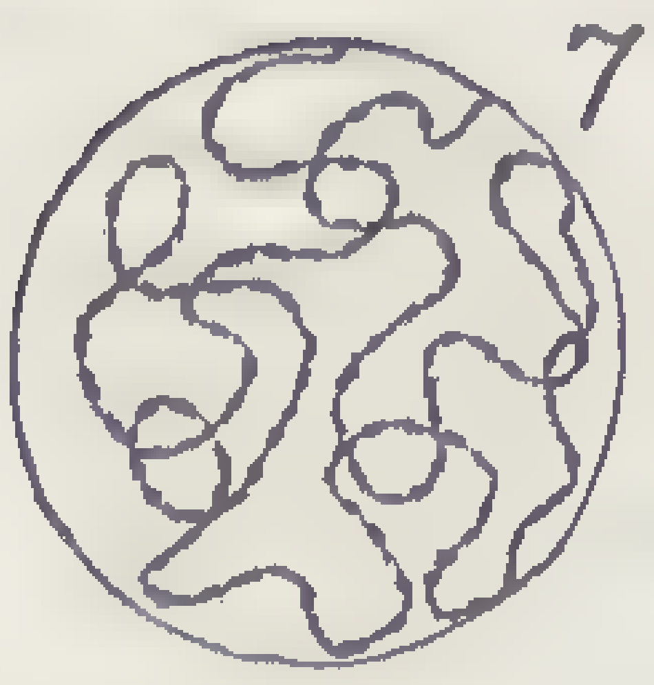
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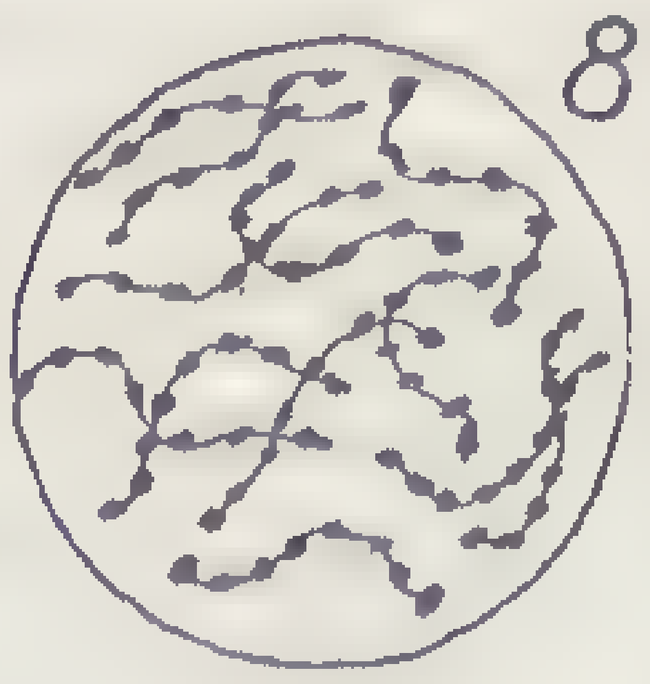
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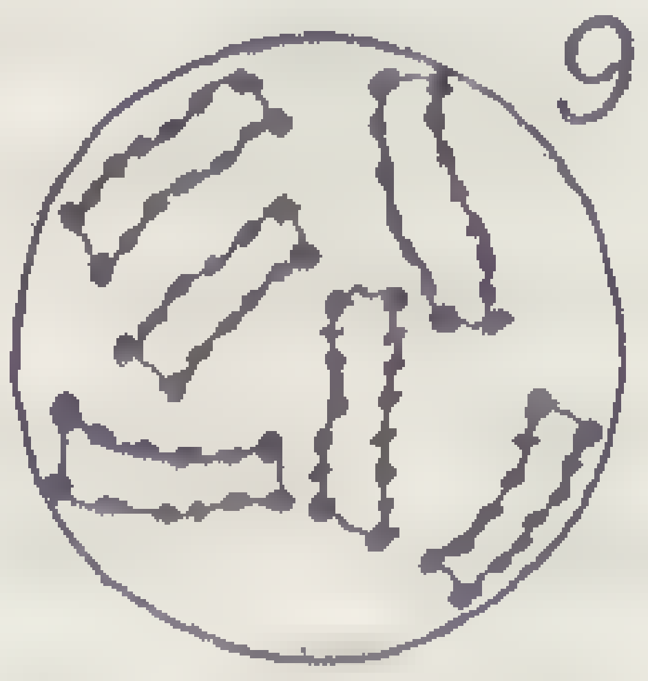
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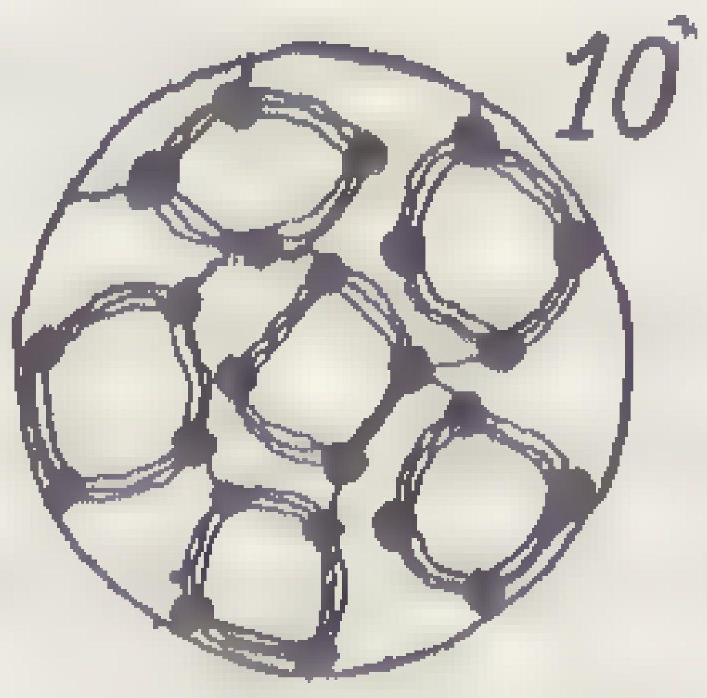
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The history of the spermatocytes of the first generation is as follows. (Compare Diagrams 3 to 6 and 7 to 10.) In the earliest prophase that I have seen (Plate III. Figs. 110, 111) the chromatic substance consists of numerous small granules, already arranged along a thread of substance, which itself stains somewhat (Diagram 3). The chromatic granules gradually become collected at twenty-four points on the thread (Diagram 4). The thread then breaks transversely into twelve segments (Diagram 5). Each of these segments has the form of a dumbbell, i. e. consists of two terminal chromosomes connected by a thread, composed of numerous linin fibres (Plate IV, Fig. 187, Plate V. Figs. 242, 243).

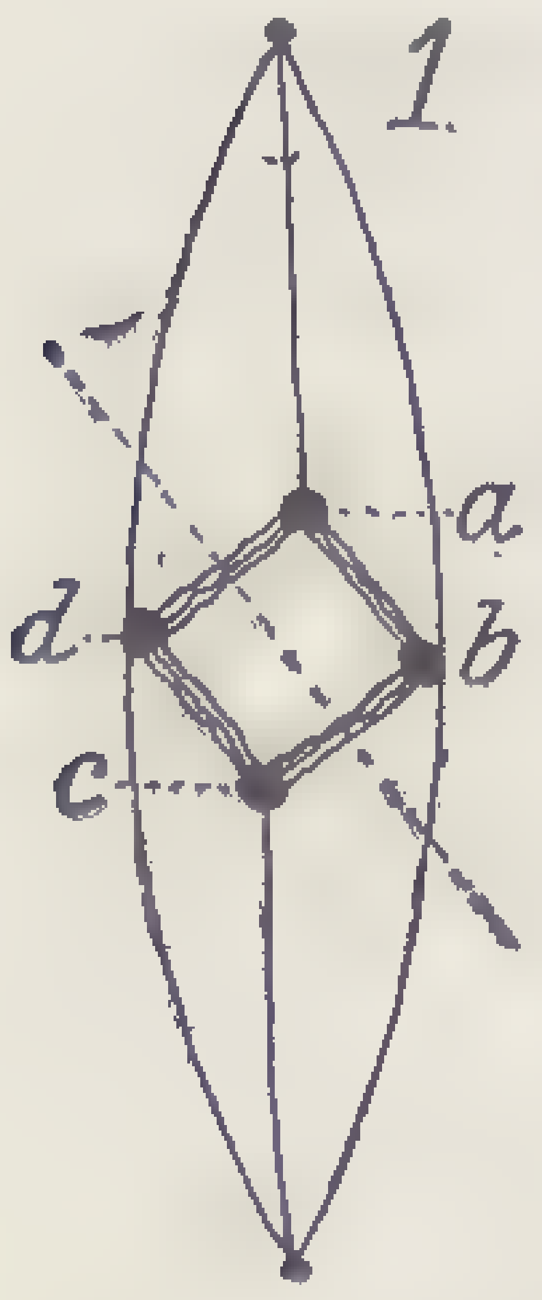
The dumb-bell figures become associated in pairs (Plate V. Figs. 229, 242, 243). Each of the six groups (Diagram 6) thus formed has the value of four chromosomes, each dumb-bell being equal to two chromosomes (Plate IV. Fig. 178, Plate V. Figs. 242, 243). These quadrivalent groups may be formed by the approximation of the pairs of dumb-bells in one or the other of two ways. Either they become arranged quite irregularly (Plate III. Fig. 116, Plate V. Fig. 229), or the pairs may at first lie across each other at right angles, and later come to be parallel (Plate V. Figs. 229, 242, 243). A comparison of the drawings last mentioned will show how by the fusion of the ends of the two parallel dumb-bells a ring results, such as is shown in Plate IV. Figs. 178, 179, 181.

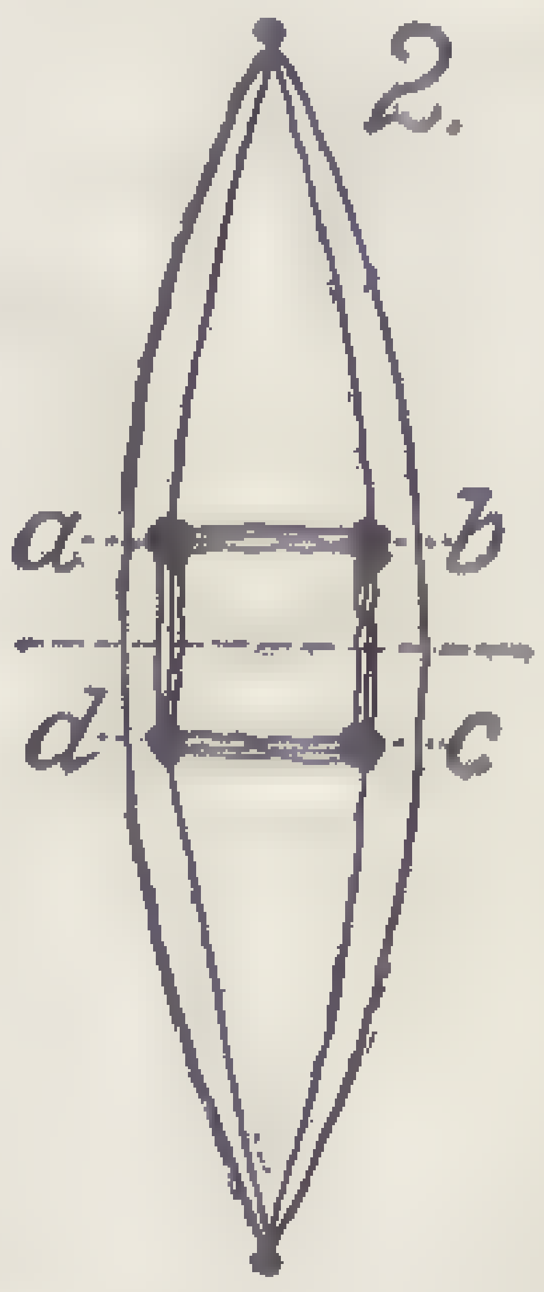
By a slight variation in the time at which the massing of the chromatin granules takes place the process up to this point may pursue a course apparently quite different from that described. The chromatin granules of the original chromatin thread do not become massed into definite chromosomes as early as in the method just outlined. Consequently the transverse divisions result in the formation of twelve segments (Diagram 8) with very irregularly serrated edges. These segmenta associate themselves (Diagram 9) in pairs (Plate V. Figs. 201, 225, 227). They are either so closely applied to each other as to appear like single rods, or else show two rows of granules (Fig. 227), and thus give the same appearance that would have resulted from a longitudinal splitting of a single segment. The component halves of these six segments separate from each other except at their ends, and thus form rings, as in Plate IV. Fig. 174. The granules scattered along these rings then collect into four chromosomes (Diagram 10). The result is, therefore, the same as by the process first mentioned.

This account of the formation of rings varies somewhat from those of vom Rath ('93) and Häcker ('93), and is entirely different from Brauer's ('93) account. These differences, as well as the points of agreement, will be discussed under the literature of the subject.

The position of the chromatic rings at the equator of the spindle is shown in Plate V. Figs. 192–195 and 237–241. The rings are always complete at this stage, and the first step in the metakinesis of the spermatocytes consists in a separation of the rings into half-rings. With the iron-hæmatoxylin method the majority of the spindles present the appearance of Figures 194, 195. The planes of the rings all pass through the axis of the spindle. Hence it is impossible to see that the chromosomes are arranged in rings, except when the rings are turned broadside toward the observer, as sometimes happens (Figs. 237–239). Figure 192 shows four rings seen from the edge and two from the side. The four chromosomes of a group may be arranged in a square rather than in a circle (Plate IV. Figs. 175, 176).

The division of the rings may present different appearances according to the position of the chromosomes with reference to the poles of the spindle. The group may be a square, with one side turned toward each pole of the spindle (Diagram 2), or it may be more diamond-shaped (Diagram 1) with an angle directed toward each pole. In either case division takes place as indicated by the dotted line, and the chromosomes *a* and *b* go to one pole, *c* and *d* to the other. The final result, therefore, is the same as before. But in the first case the chromosomes *a* and *b*, still held together by linin threads, move toward the pole maintaining unchanged their relative positions, i. e. the rod with a chromosome at either end remains at right angles to the polar axis of the spindle, and is therefore in proper position for the second division, which follows directly upon the first, and is at right angles with it, *a* going to one spermatid, *b* to the other. By the second mode the pair *a*, *b* starts for the pole, either in a very oblique position or nearly parallel to the polar axis, and with *a* in advance. It therefore must turn 45° or more so as to be in the proper position for the second division.





The later stages of the first spermatocyte division are shown in Plate III. Figs. 112, 113, 117, 118, 122, and 123, which are drawn from preparations stained with safranin and victoria-green, or by Henneguy's method. These methods do not bring out the individual chromosomes at this stage. Figures 118 and 122 show the interzonal filaments still bridging over the space between the already separated cells. Some cells at this stage (Figs. 113, 117) have a peculiar appearance, as if the division were amitotic. But the interzonal filaments between the two chromatic masses show it to be a mitotic division.

The second division of the spermatocytes is shown in Plate III. Fig. 128, and Plate V. Figs. 190, 191, 231. This division is accompanied by the formation of a typical spindle and centrosomes; it effects a separation of the constituent chromosomes of each chromatic dumbbell, and therefore results in giving each spermatid six univalent spherical chromosomes, such as are shown in Plate III. Figs. 125, 126.

The number relationships of the chromosomes in the spermatogenesis of Caloptenus may be thus tabulated : —

Spermatogonia	12 univalent chromosomes.
Spermatocytes, 1st order	6 quadrivalent chromosomes.
Spermatocytes, 2d order	6 bivalent chromosomes.
Spermatids	6 univalent chromosomes.

Expressed in individual chromosomes : —

Spermatogonia	12	Spermatocytes, 2d order .	12
Spermatocytes, 1st order .	24	Spermatids	6

Spermatids immediately after the second spermatocyte division are shown in Plate III. Figs. 125, 129, and Plate V. Fig. 232. There is at first no nuclear vacuole surrounding the six small spherical chromosomes, which are closely packed together, and immediately surrounded by the granular cytoplasm (Plate III. Figs. 125, 126, 129). The interzonal filaments are still to be seen, forming a striated body, probably the beginning of the "Nebenkern," as suggested by Platner ('86).

Some of the spermatids stained by Henneguy's method, and nearly all of those stained by Heidenhain's method, show a spherical body near the chromatic mass (Plate V. Figs. 232–235), and this body becomes included in the nuclear vesicle when a membrane is formed (Plate IV. Figs. 140, 141, Plate V. Figs. 232, 236). I regard this body as the centrosome which is left in each spermatid after the last spermatocyte division, and I also believe it to be identical with the very conspicuous body which forms the neck of the spermatozoön (Plate V. Figs. 196–200). The chromatic substance fuses into a smoothly contoured mass, which soon assumes the crescent shape so common in insect spermatogenesis. The neck-body lies within the nuclear membrane opposite the concavity of the chromatic crescent (Figs. 198–200). The chromatin undergoes chemical and physical changes during the metamorphosis of the spermatid, but the neck-body remains practically the same in size, and does not alter its affinity for stains. It becomes the neck of the spermatozoön (Plate IV. Figs. 139–158, Plate V Figs. 196–200). The chromatic crescent is at first less dense, and stains less deeply; then it becomes concentrated, and stains nearly black by Heidenhain's method. These changes in density are not well shown in the figures. At the same time it becomes elongated, one end applying itself to the neck-body, the other becoming the tip of the spermatozoön head.

The nuclear vacuolation, much reduced, persists for some time near the neck-body (Fig. 196), then disappears entirely, and the further metamorphosis of the head consists largely in an elongation. By Henneguy's method the neck-body is only rarely stained. This explains its absence in Figures 142–154. By Heidenhain's method it becomes in all spermatids a very conspicuous black spherical body (Plate V. Figs. 196–200).

I pass now to a consideration of the results reached by other students of spermatogenesis in insects.

Sabatier ('90) has discussed in a short article the spermatogenesis of the Locustidæ. The conclusions to which he comes are rather startling. In regard to the metamorphosis of the spermatid, he says: "Près du noyau mais non à son contact immédiat apparait dans le protoplasme une vésicule sphérique . . . la vésicule protoplasmique." Sabatier maintains that there is an almost total degeneration of the nucleus, but admits that it gives rise to the "Kopfkappe": "Cette dernière dérive donc du noyau et fournit un exemple remarquable de la dégénérescence ou altération du noyau de la cellule spermatique." It is quite remarkable that the nucleus is able to form only the Kopfkappe when, presto, "cette vésicule devenue fusiforme et vivement colorable constitue ce que l'on considère comme la tête du spermatozoïde."

I have never seen any such nuclear degeneration, nor any extranuclear vesicle of such paramount importance. The head of the Caloptenus spermatozoön arises from the six chromosomes inherited by each spermatid.

Blochmann ('87) describes the formation of the polar bodies in Blatta germanica.

The work of vom Rath ('91[a] and '92) on the spermatogenesis of Gryllotalpa must receive special notice. My account of the spermatogenesis of Caloptenus confirms a large part of vom Rath's results, but differs from his in several points, and suggests another interpretation of the last two divisions. What these differences are will soon be apparent.

Vom Rath ('93) has already called attention to the many groups of animals in whose spermatogenesis or oögenesis ring formation and Vierergruppen have been observed by different authors; but with one exception (Flemming, '87) he mentions those authors only who have noticed and remarked about these chromatic figures. It may seem venturesome, but I wish to suggest the same interpretation for the figures of various authors who either had no definite idea of Vierergruppen, or considered the conditions exhibiting them abnormal (Flemming, '87), and were thus unprejudiced in favor of rings or Vierergruppen in making their drawings. The older works will be mentioned first, and the important works of Boveri ('90), Brauer ('93, '94), Häcker ('93), Henking ('90, '91, '92), and vom Rath ('91^a, '92, '93), will be considered later.

Flemming ('87, pp. 444, 445) saw Vierergruppen in the Salamander. Figures 46–50 of his paper show chromosomes arranged in groups of four, the groups being scattered quite irregularly over the spindle, much as vom Rath figures them in his latest paper ('93). Flemming considered this arrangement as abnormal: "Sie [the grouping into fours] kann wohl in der That als eine Anomalie bezeichnet werden, obwohl ich noch nichts darüber weiss ob aus den Folgestadien etwas normales werden kann oder nicht, . . . es finden sich also Gruppen von je vier Kügelchen von denen je zwei aneinanderhängen. Diese liegen anscheinend ganz regellos über die ganze Spindel hingestreut, nur offenbar mit der Tendenz sich nach den Polen anzuhäufen." Vom Rath calls attention to Flemming's explanation of these figures, and holds, quite rightly, that the groups are moving, not as Flemming imagined, toward the poles, but toward the equator, there to be separated into bivalent dumb-bells. Flemming believes he finds a tendency to irregularity in those spindles which bear four-grouped chromosomes, and considers such irregular spindles as so many stages in the degeneration of a bipolar spindle into a tripolar one. If with Flemming it is denied that the groups of four occur in the regular course of development, it must be concluded that these are degeneration stages.

Platner ('86) has figured in Helix pomatia several stages of rings and their division without so interpreting them. Figure 4 of his article "Ueber die Entstehung des Nebenkerns," etc., shows very clearly the ring condition previous to division. In his Figure 5 are groups of four chromosomes. Figure 12 shows rings on the equator of a spindle, and Figures 15–17 are metakinetic and dyaster stages, in which the spherical chromosomes are coupled into dumb-bell figures and some of the dumb-bells have rotated 90° and are ready for the second division, just as I have seen them in Caloptenus.

I would call attention also to the following cases drawn from the literature of the subject: La Valette St. George ('85, Figs. 16, 17, '86, Figs. 11, 21, 22), Zacharias ('87, Taf. VIII. and IX.), Kultschitzky ('88, Fig. 3, and '88^a, Figs. 16, 17, 22), Carnoy ('85, '86, and 86^a), Guignard ('91), Baranetzky ('90, Figs. 23, 26, 40), Hermann ('89, Fig. 31), Lukzanow ('89, Figs. 21, 23), Henking ('92, Figs. 101, 153, 190, 216, 217, 229, 413, 418), Stuhlmann ('86, Figs. 228, 233), and Moore ('93, Fig. 1).

The terminology which I have used is that of La Valette St. George, as adapted by Boveri : —

Spermatogonium = Hertwig's Ursamenzelle.
Spermatocyte, 1st order = " Samenmutterzelle.
Spermatocyte, 2d order = " Samentochterzelle.
Spermatid = " Samenenkelzelle.
Spermatozoön.

G. W. Field ('93) uses a terminology which seems to admit one less spermatocyte stage than is recognized by *authors generally :* " We find that the largest cells, the spermatogones (using the nomenclature proposed by La Valette St. George and now very generally adopted), divide by mitosis and form two spermatocytes. Next each spermatocyte divides, *also by mitosis,* forming two spermatids. Each spermatid then changes directly into the spermatozoön, without further division. Thus each spermatogone gives rise to four spermatids." Field uses this apparently as a general scheme of spermatogenesis. " Spermatogones" are, I suppose, spermatogonia. But they are not "the largest cells" in Boveri's scheme, nor do I find that La Valette St. George or any other author has applied the term to these large cells, which Boveri designates as spermatocytes of the first order. The spermatogonia after they have ceased dividing as spermatogonia become by a process of growth spermatocytes (Boveri's spermatocytes, 1st order). Field's "spermatogones" therefore probably correspond to Boveri's spermatocytes of the 1st order, his "spermatocytes" to Boveri's spermatocytes of the 2d order, and the spermatogonia of Boveri are unmentioned. Field has therefore extended the use of the term spermatogonia to cover the whole period of that cell generation which Boveri calls at its beginning the last generation of spermatogonia, and during the rest of its existence spermatocytes of the first order ; consequently he designates as a *spermatogonium division* one that Boveri calls a *spermatocyte division.* It is difficult to see why the fact that " each spermatocyte divides *also by mitosis*" need be so strongly emphasized. It would be much more strange if the spermatocytes divided amitotically (compare vom Rath '91 and '93, and Ziegler '91).

Henking ('91) in his paper on Pyrrhocoris has considered the origin and fate of the chromatic rings. His Figures 13–20 show stages in the formation of the rings. Henking differs from most other authors in denying that there is any doubling of the chromosomes between the last division of the spermatogonia and the first division of the spermatocytes. He maintains that the first division of the spermatocytes is a reduction division and the second an equation division. His number relationships for the chromosomes are hence the following: —

Spermatogonia	24
Spermatocytes, 1st order, 12 *bivalent rings*	24
Spermatocytes, 2d order	12
Spermatids	12

The only reference by Henking to rings of the value of four chromosomes is in this sentence: "Ich mache besonders auf die mit vier Verdickungen versehenen Ringe 1 und 2 in Fig. 20 aufmerksam." The two rings to which Henking refers contain each four nearly spherical chromosomes, and these, I believe, are the only instances in which Henking recognized the real value of chromatic rings. Each ring contains four chromatic elements, each half-ring two elements, and since these two elements are separated from each other at the second spermatocyte division, this, contrary to his conclusion, is just as truly a reduction division as is the first. But Henking objects to this interpretation: "Es findet hier also keine Reduction statt, sondern eine gewöhnliche Aequationstheilung, welche jedoch hier schon von fernher vorbereitet war." But if each ring has the value of four, not simply two, chromosomes, the same argument could be applied to the first as well as the second spermatocyte division, as Brauer ('93) has already done. The soundness of these objections will be considered in connection with Brauer's paper.

Häcker ('92^{a} and '93) has seen ring formation and Vierergruppen in the oögenesis of several marine Copepods. In the genera Euchæta, Calanus, Cyclops, Diaptomus, Canthocamptus, and Heterocope, he maintains that "zwischen die letzte Theilung der Ureizellen und die erste Theilung der Reifungsphase ist kein feinfadiges Ruhestadium des Kernes (Keimbläschenstadium) eingeschaltet." In the eggs of some females this resting stage is passed over, in others not. In those females in which the resting stage in oögenesis is twice omitted, i. e. both before and after the formation of the first polar globule, Häcker ('92) suggests, as a motive for the omission of the first resting stage, that in this way "im Mikrokosmus des regenerativen Lebens eine weitgehende Anpassungsfähigkeit zur Geltung gelangt." This omission, then, is a biological adaptation. The maturation of the egg is thus brought about sooner. This explanation is mentioned, because it has a direct bearing upon any interpretation of the rings, as will soon be seen. Häcker

('93, pp. 462, 463) describes the formation of the Vierergruppen as follows: "Die eigentliche chromatische Substanz konzentriert sich nämlich zunächst auf bestimmte Stellen der Doppelfadenschlinge, und an jeder dieser Stellen tritt mehr und mehr eine scharfe Knickung hervor (Fig. 15 *a*, 16 *a*), durch welche diese Doppelfadenpartieen je in zwei gleiche Schenkel getheilt werden. . . . In einem weiteren Stadium findet eine tropfenförmige Verdickung der 4 Enden der Doppelschenkel statt (Fig. 16 *b* und *c*), in den Ecken der Doppelwinkel kommt es dann zur Zerlegung derselben (Fig. 16 *d*), die vier Schenkel verkürzen sich noch mehr und das Resultat dieser Veränderungen sind demnach Bündel von je vier kurzen dicken Stäbchen, welche in der oben angegebenen Weise durch feine Doppelfäden mit den Nachbarbündeln verbunden sind. Es sind die charakteristische Vierergruppen welche immer wieder und wieder vor der ersten Theilung der Reifungsphase auftreten." Häcker's theoretical explanation of the Vierergruppen may be learned from the following: "Wenn wir unter einem Paar von Schwesterelementen ['identischen Idanten,' Weismann '91] solche Elemente verstehen welche durch Längsspaltung eines Mutterelementes entstanden sind, so besteht also jede Vierergruppe aus zwei Paaren von Schwesterelementen welche im zusammenhängenden Doppelfaden ursprunglich hintereinander gelegen sind." Häcker ('92) had previously, in considering the longitudinal splitting of the chromatic thread in the prophases of the first division, treated this as a process by itself, and so had regarded both polar-body divisions as reduction divisions. But later he came to view this splitting as a precocious preparation for the formation of the first polar body, or rather as a process pushed back in time by the subsequent introduction of a germinative-vesicle condition. He now ('93) sees in the first division a *modified* equation division: "Um zu beweisen dass die erste Theilung eine modificierte Aequationstheilung ist, müssten wir zeigen dass ihr eine einmalige Längsspaltung vorangeht durch welche die Normalzahl der Elemente verdoppelt wird, und dass dann bei der Theilung die so erzeugten Schwesterelemente auseinandertreten."

Häcker's conclusion with regard to the Vierergruppen is, in his own words: "Heissen die im Chromatinfaden hintereinanderfolgenden Idanten *a*, *b*, *c* . . ., so würde der längsgespaltene Chromatinfaden sich nach Weismann durch $\begin{Bmatrix} a\ b\ c\ .\ .\ . \\ a\ b\ c\ .\ .\ . \end{Bmatrix}$ darstellen lassen, und die Formel für eine Vierergruppe ist: $\begin{Bmatrix} a & b \\ \acute{a} & b \end{Bmatrix}$. *Jede Vierergruppe besteht also im*

Sinne Weismann's aus zwei Paaren von Schwesterelementen und nicht, wie dies nach Boveri's und Brauer's Angaben der Fall sein würde, aus vier Enkelelementen $\left(\left\{\begin{matrix} a\ a \\ a\ a \end{matrix}\right\}\right)$."

The separation of the sister elements, which according to Häcker occurs in the first division, constitutes an equation division, and "In der zweiten Richtungstheilung erfolgt dann die definitive Trennung der nichtidentischen Idantenpaare." Therefore, the second division alone is a reduction division.

Vom Rath ('91^a, '92, '93) has in two important papers discussed the formation of rings and the meaning of the Vierergruppen in connection with the question of reduction. Häcker and vom Rath agree in all essential points as to the origin of the rings, as can be seen from the following quotation from vom Rath's last paper: "In allen von mir untersuchten Fällen der Spermatogenese und Ovogenese entstehen die Vierergruppen vor der Reifungsperiode in gleicher Weise dadurch, dass im Knäuelstadium zwei hintereinander gelegene Segmente mit einander verbunden bleiben und mit den durch die Längsspaltung des Chromatinfadens entstandenen ebenfalls verbundenen zwei Schwestersegmenten eine bald innigere (Ringbildung) bald losere (keine Ringbildung) Zusammengehörigkeit bewahren. Aus jedem dieser vier Segmente entstehen dann durch Kontraction vier Stäbchen — oder Kugelchromosomen. Es scheint mir daher das Natürlichste zu sein, jede Vierergruppe als aus vier Einzelchromosomen bestehend anzusehen." Häcker's account, as previously quoted, is strikingly similar to this. Vom Rath evidently meant to say that out of each of the four segments arises by contraction *one* spherical chromosome, *not four*. If four chromosomes arose from *each* of the four segments, we should have groups of sixteen.

Vom Rath inclines to the belief that both maturation divisions are reductions: "Wieder andere, nämlich Weismann, Häcker und ich, lassen die Reduction durch beide Theilungen erfolgen." He adopts Häcker's formula $\left(\left\{\begin{matrix} a,\ b \\ a,\ b \end{matrix}\right\}\right)$, quoted above, for the Vierergruppen. The scheme of numbers of chromosomes, exactly the same as that of Häcker, is as follows: —

For Gryllotalpa.	For Salamandra.
Spermatogonia, 12.	24.
Spermatoc. 1st order, 6 rings, 24 chromosomes.	12 rings = 48 chromosomes.
Spermatoc. 2d order, 6 half-rings, 12 "	12 half-rings = 24 "
Spermatid, 6 chromosomes.	12 chromosomes.

The number of chromosomes in the spermatocytes of the first order is thus double the normal number, and this is reduced to one half the normal number by two reduction divisions (vom Rath, '92 and '93).

Brauer ('93) in studying the spermatogenesis of Ascaris has come to some quite definitely stated conclusions in regard to the meaning of the reduction of the chromatin, which can be made clear by a few quotations: —

"Es ist zwischen beiden [spermatogonia and spermatocytes] ein echtes Ruhestadium des Kerns vorhanden, von einem Fortbestehen der Chromosome der Spermatogonien kann keine Rede sein."

"Es bedarf kaum einer näheren Auseinandersetzung, dass diese Ansicht sich mit der Individualitätshypothese der Chromosome . . . nicht verträgt. Die Chromosome sind für mich keine selbständigen Individuen, sondern sie sind nur die Verbände für die zahllosen kleinen Chromatinkörner, welchen allein der Werth eines Individuums zukommt."

"Ist meine Ansicht über die Bedeutung der Chromosome richtig, so erfolgt bei diesen Theilungen keine Reduction der Zahl der Chromatinkörner, sondern nur eine solche ihrer Masse. Eine Reductionstheilung im Sinne Weismann's findet mithin nicht statt. Eine solche dürfte meiner Ansicht nach überhaupt unmöglich sein, wo wenigstens die Theilung auf karyokinetischem Wege erfolgt." A like conclusion is reached for the *centrosomes:* "So müssen dieselben bei der Befruchtung in irgend einer Weise eine Reduction ihrer Masse erleiden, da sonst eine stete von Generation zu Generation fortschreitende Vergrösserung eintreten müsste."

Speaking of the longitudinal splitting of the chromatin granules, he says: "Da diese Theilung aber das Wesen der ganzen Karyokinese ausmacht, so halte ich alle diejenigen Erscheinungen welche ihr folgen, wie das Ansammeln der Körner auf wenigen Fäden, ihre Vereinigung zu grösseren Körnern, der Zerfall eines Fadens in Segmente und schliesslich in Chromosome, für weniger bedeutend." Briefly, Brauer does not believe in any reduction division in the Weismannian sense of the term, but only in a reduction of the *mass* of the chromatin.

Thus there have been proposed by different authors four different solutions of the question of reduction. Henking holds that the first maturation division is a reduction, the second an equation division; Hertwig considers the first an equation and the second a reduction division; Brauer maintains that there is no "reduction" in either division (except in mass), whereas Weismann, Häcker, and vom Rath maintain two reduction divisions. But Häcker ('93) now calls the first a "modified equation" division, and only the second a reduction division.

I have used the word "reduction" without indicating the particular sense in which I use it. The definition of reduction proposed by Weismann ('92), and adopted by vom Rath, Häcker, and others, is that which I prefer, and according to which I have used the term. This is: "Unter Reductionstheilung verstehe ich eine jede Kerntheilung durch welche die Zahl der Ide welche im ruhenden Kern vorhanden war, für die Tochterkerne auf die Hälfte herabgesetzt wird." It is not necessary to adopt Weismann's terms, "ids, idants," etc., in order to use his definition. If the developmental possibilities are only one half as great in the daughter nucleus as in the mother nucleus, there has been a reduction in Weismann's sense. If a nucleus contains four elements which happen to be two pairs of identical elements, the formula would be

$$\begin{array}{ccc} & n & \\ x & \begin{array}{c|c} a & a \\ \hline b & b \end{array} & y. \\ & m & \end{array}$$

Now, if the division takes place along the line *x y*, there is a reduction in Weismann's sense; but if the division be along the line *m n*, it is an equation division. Either division would reduce the chromatic *mass*, but only the first would reduce the number of *different* elements (ids) in the daughter as compared with the mother cell.

Since the rings, or Vierergruppen, have already been found in the oögenesis and spermatogenesis of numerous species in different groups, this arrangement of the chromatin just before the maturation divisions is certainly very general, if not practically universal. In order, therefore, to interpret properly these two divisions, and to come to any sound conclusions with regard to the reduction question, it is of fundamental importance carefully to study the formation of the Vierergruppen. Häcker ('93) and vom Rath ('93) have already called attention to the fact that the double longitudinal splitting of the chromatic thread, maintained by Boveri and Brauer, must bring about groups of four *identical elements.* The formula for a Vierergruppe would then be $\left\{\begin{matrix} a & a \\ a & a \end{matrix}\right\}$. There could not in this case be any Weismannian reduction in either division, for there is only one *kind* of *id* in all the four elements of the group. If the Vierergruppen always arose as Brauer describes the process, — i. e. by two longitudinal splittings of the *chromatic granules,* which alone, he believes, possess an individuality, — then the four components of each group would be identical, and there could be no reduction division. Brauer therefore contends that a Weismannian reduction is impossible in karyokinetic division, and indeed never occurs. For Brauer the reduction question has consequently found its final solution: There is no reduction except merely one of mass. This would offer a very simple answer to the problem. But Henking ('90, '91, '92), Rückert ('92, '93), Häcker ('91, '93), and vom Rath ('91^a, '92, '93) have been able to see only *one* longitudinal splitting. I have seen no evidence of *any* such splitting of the chromatic thread in Caloptenus. Since coming to that conclusion, I have read in Born's paper on the egg of Triton ('94) that he finds a doubling in the number of the chromatic elements during the germinative-vesicle condition, but this doubling does not result from a longitudinal splitting. The chromatic thread divides transversely into twice as many segments as there were chromosomes in the Ureizelle. Born's statement that "eine Verdoppelung durch Quertheilung stattfindet" is in essential agreement with my results.

A remark by Wagner ('92) — to the effect that twice as many chromosomes arise during the resting stage immediately before the first maturation division as were in the cells of the preceding generation, but that this does not imply any such definite longitudinal splitting as Brauer and other authors maintain — may also be interpreted as in harmony with my conclusions.

Since the chromatin in the resting stage is very finely divided, — at least into finer particles than the "Chromatinkörner" to which Brauer ascribes the dignity of individuality, — it seems to me just as arbitrary to consider these homogeneous "Chromatinkörner" units, which by division must give rise to identical daughter grains, as to make the same supposition with regard to the chromosomes.

But further, even if we grant for a moment that the Vierergruppen do arise by two longitudinal divisions of the chromatic granules, what evidence have we that each Vierergruppe consists of four identical elements? Brauer maintains that both these splittings take place very early in the resting stage. The granules are extremely small. Each chromatic quarter of the group increases considerably in size. This growth takes place while they are separated (held together only by linin threads). There is still the probability that chromatic substance is formed in the nucleus during the process, and becomes associated with the substance of the Vierergruppen. In order to insure the identity of the elements of a Vierergruppe, two longitudinal divisions must take place after all growth of the chromatin has ceased, and we must at the same time assume that the chromatic elements are homogeneous, or if they are not homogeneous, that there is an exact halving of the component particles of the elements of the Vierergruppe. But Brauer considers the four elements of a group identical because they all arise, by two divisions, from one.

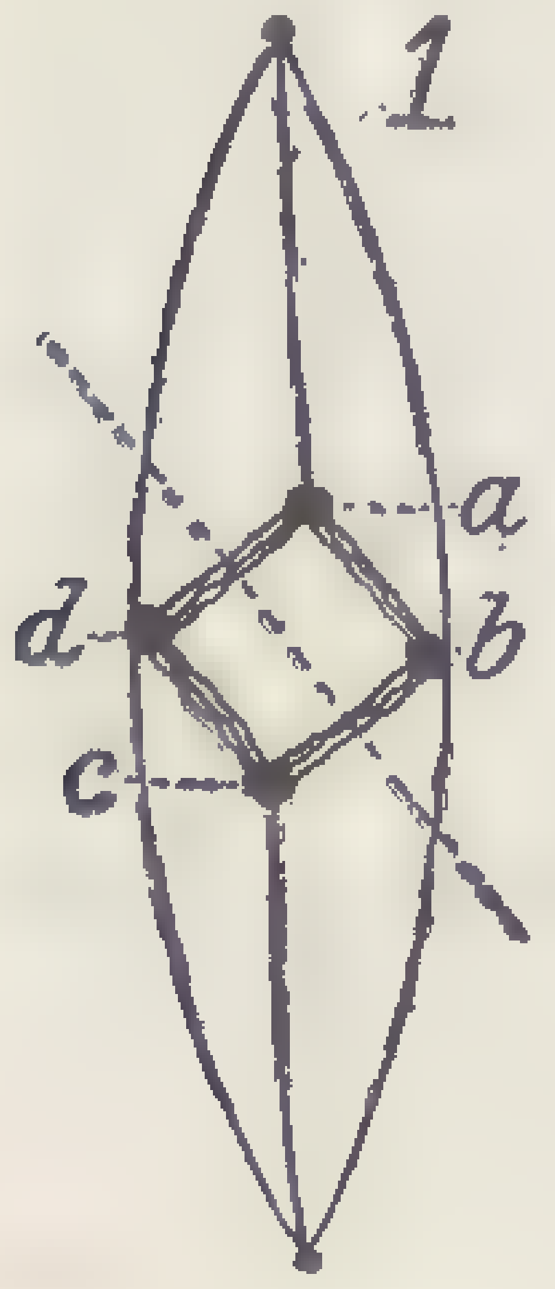
Again, if this whole process be only to secure a reduction of the *mass* of chromatin, the doubling of the chromatic elements, and the long, laborious process of mitosis would be unexplained and unjustified, as Weismann has pointed out; for a halving of the mass could be brought about by amitotic division. According to Weismann, the formula for Brauer's Vierergruppe would be (Häcker, '93) $\begin{Bmatrix} a & a \\ a & a \end{Bmatrix}$. We start with one element, a; this undergoes two longitudinal splittings, and then two separations by the two maturation divisions, and we then have just what we started with. The series formulated would be

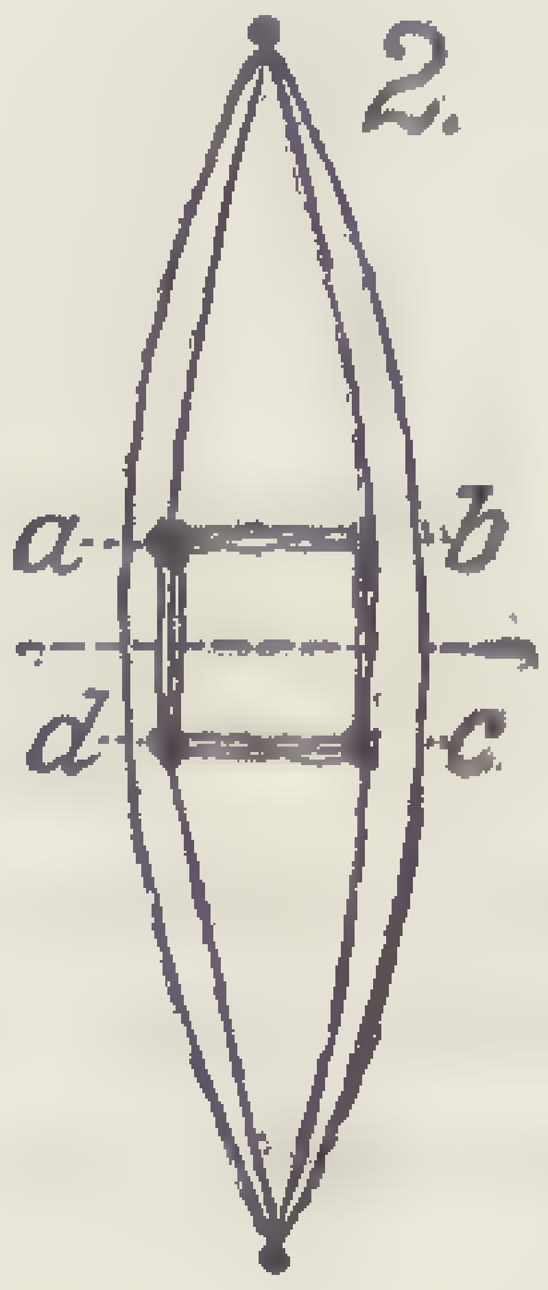
$$a \qquad \begin{matrix} a \\ a \end{matrix} \qquad \begin{matrix} a & a \\ a & a \end{matrix} \qquad \begin{array}{c|c} a & a \\ a & a \end{array} \qquad \begin{array}{c|c} a & a \\ \hline a & a \end{array}.$$

But Henking, Weismann, Häcker, Rückert, vom Rath, and others allow only one longitudinal splitting, and their formula for the Vierergruppen, as stated by Häcker ('93), and accepted by vom Rath ('93), is $\begin{matrix} a & a \\ b & b \end{matrix}$. This evidently permits only one reduction division in the Weismannian sense. Vom Rath and Weismann are therefore inconsistent when they hold to a longitudinal splitting in the spirem condition, and yet consider both maturation divisions as reductions. If the Vierergruppen have the formula $\begin{Bmatrix} a & a \\ b & b \end{Bmatrix}$, there are but two sorts of ids, a and b, and it is simply impossible to get more than one reduction division. If from the nucleus $\begin{matrix} a & a \\ b & b \end{matrix}$ arises by division two nuclei, $\begin{matrix} a \\ b \end{matrix}$ and $\begin{matrix} a \\ b \end{matrix}$, this is by Weismann's own definition an equation division, and only when these two cells become by division the four ultimate products of maturation a, b, a, b, can we speak of a reduction.

Häcker at first considered both divisions as reductions ('92), but later ('93) he rightly came to the conclusion that the longitudinal splitting in the spirem stage was a preparation for one division, and that the final separation of the sister elements thus produced constitutes an equation division, — a "modified equation division," he calls it, because the splitting, which ordinarily occurs at the equator of the spindle is here precociously introduced in the spirem condition.

According to my interpretation of the Vierergruppen in Caloptenus, the formula would be $\left\{\begin{matrix} a\ b \\ c\ d \end{matrix}\right\}$. Both the divisions following the formation of a Vierergruppe would therefore be reductions, and it would be quite immaterial whether the first division gave rise to two cells *a b* and *c d*, or to the two cells *a c* and *b d*. In Caloptenus the rings may be placed upon the spindle equator in either of the two positions represented in Diagrams 1 and 2.

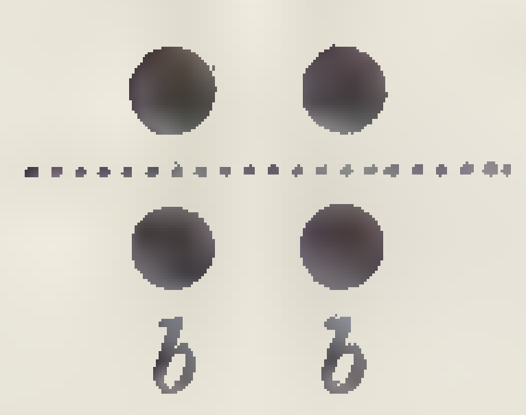




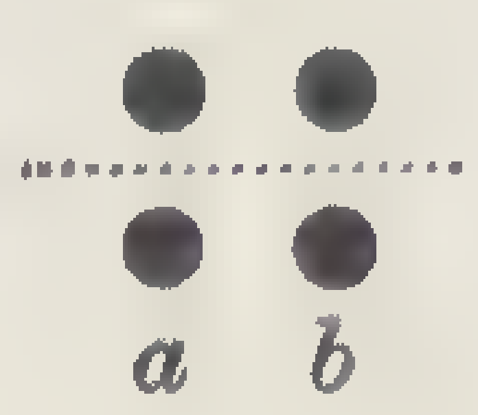
This offers, perhaps, an explanation and reconciliation of the contradictory views of Henking, Hertwig, Häcker, and others. As has been said, Henking holds that the first division is a reduction division, and the second an equation division, while most authors make the first an equation, and the second a reduction division. Henking ('91) did not, in his Pyrrhocoris paper, recognize the existence of Vierergruppen as a regular stage in maturation; but I feel justified by his Figure 20 in believing that they were really present in Pyrrhocoris, just as in Gryllotalpa, Caloptenus, etc. Now, supposing the proper formula for the Vierergruppen to be $\left\{\begin{matrix} a\ a \\ b\ b \end{matrix}\right\}$, why might it not happen in different nuclei, or in different chromatic groups of the same nucleus, that one group should divide thus, $\frac{a\ a}{b\ b}$, and another thus, $\begin{array}{c|c} a & a \\ b & b \end{array}$? Henking must assume that all the groups are arranged on the spindle so as to separate the non-identical idants by the *first division.* Häcker says: "In der *zweiten* Richtungstheilung erfolgt dann die definitive Trennung der nichtidentischen Idantenpaare."

The following diagrams may illustrate these positions: —

I.



II.



III.

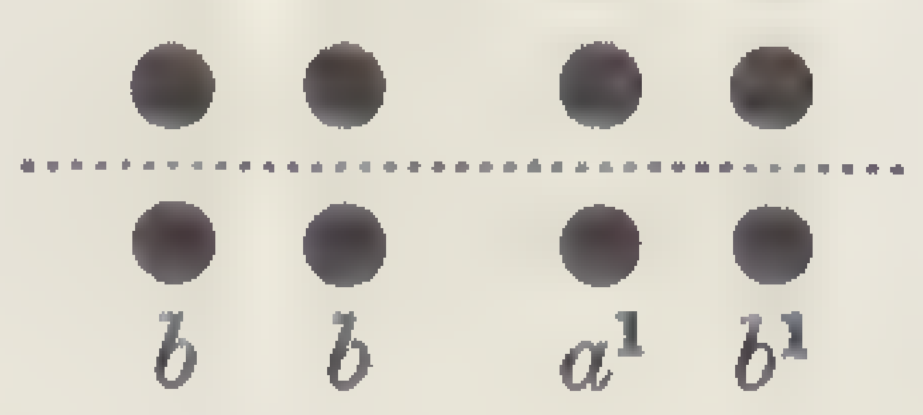


Diagram I. illustrates Henking's view of the first division interpreted according to the scheme of the Vierergruppen. All the groups would thus suffer reduction division.

Diagram II. serves to represent the view of Häcker, Weismann, vom Rath, and others. The first division is here seen to be an equation division. But none of these authors has offered any reliable criterion by which we may judge whether it is the "sister idants" or the "non-identical idants" that are separated by the first division. They have presented no satisfactory evidence that in the same nucleus all groups undergo either a reduction division or an equation division. How are we certain that one group does not undergo a reduction division at the same time that another in the same nucleus passes through an equation division? This possibility, as shown in Diagram III., is not excluded. Häcker ('92, Fig. 22, and '93, Fig. 116) believes he has seen two examples where the *non-identical* idants were still in connection with each other after separation of the sister pairs in the formation of the first polar globule, but the figures are rather unsatisfactory.

This discussion will, I hope, have made one thing clear: the absolute necessity of a knowledge of the *origin* of the Vierergruppen, in order to a proper interpretation of the reduction question. If Brauer's account of the origin of the Vierergruppen be correct, there can be no reduction. If Häcker and vom Rath have rightly described their origins, there is one, and only one, reduction. If my description of the ring formation be accurate, there may be two reductions. I am quite willing to grant that, as Brauer maintains, precocious preparations for both divisions are made in the prophases of the first spermatocyte division. But Brauer maintains an origin for the groups of four, which determines that each group shall consist of four identical elements, and thus does away with Weismannian reduction, while I contend that, owing to the manner of their origin, all four elements may be different or unlike one another, and therefore that both divisions may be reductions.

The fate of a Vierergruppe, according to the four views mentioned, may again be brought together in diagrammatic form for comparison.

Brauer:—

$$a \qquad \begin{array}{c} a \\ a \end{array} \qquad \begin{array}{cc} a & a \\ a & a \end{array} \qquad \begin{array}{c|c} a & a \\ a & a \end{array} \qquad \begin{array}{c|c} a & a \\ \hline a & a \end{array}$$

Henking:—

$$\begin{array}{c} a \\ b \end{array} \qquad \begin{array}{cc} a & a \\ b & b \end{array} \qquad \begin{array}{cc} a & a \\ \hline b & b \end{array} \qquad \begin{array}{c|c} a & a \\ \hline b & b \end{array}$$

Vom Rath, Häcker: —

$$\begin{array}{c} a \\ b \end{array} \qquad \begin{array}{cc} a & a \\ b & b \end{array} \qquad \begin{array}{c|c} a & a \\ b & b \end{array} \qquad \begin{array}{c|c} a & a \\ \hline b & b \end{array}$$

My view: —

$$\begin{array}{cc} a & b \\ c & d \end{array} \qquad \left\{\begin{array}{l} \begin{array}{c|c} a & b \\ c & d \end{array} \\ \\ \text{or } \begin{array}{cc} a & b \\ \hline c & d \end{array} \end{array}\right\} \qquad \begin{array}{c|c} a & b \\ \hline c & d \end{array}$$

As to the question of reduction in the two maturation divisions, only four suppositions can be made: (1) the first division only is a reduction; (2) the second only is a reduction; (3) both are reductions; (4) neither is a reduction. As we have now seen, all these suppositions have been made by different authors. We may well say with vom Rath, that "eine allgemein befriedigende Lösung ist nur dann möglich wenn die Autoren sich zuerst über den Begriff 'Reduction' völlig geeinigt haben."

The assertion of Häcker and vom Rath ('93), that the resting stage which immediately precedes the first maturation division is sometimes omitted, has intimate connection with the question of reduction, and its meaning, it must be admitted, is not yet entirely explained. The accounts of Häcker and vom Rath are too meagre to allow a detailed comparison of the processes in cases where there is no "Keimbläschenstadium" with those in which it exists. Perhaps we can never settle definitely the question of reduction, but *material for its solution should be sought in a careful determination of the origin and meaning of the Vierergruppen.*

Cambridge, May 16, 1894.

POSTSCRIPT.

In the Bulletin of the College of Agriculture of the Imperial University of Japan has recently appeared a paper by Kametaro Toyama ('94), "On the Spermatogenesis of the Silkworm." The author has arrived at conclusions which in part agree with my own, but in part are quite different.

Like myself, Toyama was unable to find any longitudinal splitting of the chromatic thread in the prophases of the first spermatocyte division. He gives the following account of the complicated series of movements of the chromatin during the prophases: "A nucleolus is generally seen in the network of linin and chromatin. . . . Most of the chromatin granules become collected to one side of the nucleus and form an irregular mass, . . . become again separate from each other and arrange themselves along the radiating linin fibres, and the skein stage is thus obtained. . . . The chromatin granules scattered in the nucleus become again collected in the centre of it, and present an irregular mass as before. . . . In a still later stage the chromatin granules again commence to separate from one another. . . . A little before the appearance of the centrosomes in sperm-mother-cells the chromatin granules . . . gradually collect here and there and assume ring-shaped structures."

Unfortunately the author presents no satisfactory evidence for this series of changes. He may have seen all the stages which are enumerated above, but he gives no proof that they succeed one another in the order he has stated. In the earliest prophases Toyama finds the chromatin in nearly the same condition in which I find it in Caloptenus, and just before the first maturation division he finds the chromatin arranged in quadrivalent rings. The progress toward the ring stage is, according to his account, twice interrupted by retrogressive processes. One cannot easily conceive the purpose of these complications, and the evidence for such an hypothesis could never be conclusive without direct observation of the process in the living condition. I know no reason why we might not arrange Toyama's Figures 23–43 in one continuous series. All stages represented in those figures are very young, and the numerous intermediate stages between them and Figure 44 are not shown. The concentrated condition of the chromatin seen in the author's Figure 30 seems to me due to bad preservation.

I disagree entirely with Toyama as to the processes in the maturation divisions. Toyama thinks that the Vierergruppen break up into their four elements, and that these arrange themselves around the equator of the spindle in a single row. He has figured spermatocytes with seven Vierergruppen (Figs. 44, 45), and he tells us that there are 28 separate chromosomes on the spindle of the first maturation division. We must seek his proof of this statement in his Figures 50–52. These seem to me to present seven quadrivalent rings, rather than 28 single chromosomes. In his Figure 50 Toyama has represented only 7 chromatic bodies on the spindle. Are we to suppose that there were 21 on the other side of the spindle? Our author has given no equatorial view of the spindle in which simple spherical chromosomes are arranged in one row. His Figures 50 and 51 represent what he has considered stages in the transverse division of the simple chromosomes. But I have seen in Caloptenus chromatic *rings* in such a position as to give exactly the same appearance. In Caloptenus there is no transverse division of the chromosomes in the maturation divisions. The ring does not break up into four simple chromosomes before the period of metakinesis. The separation of the ring into its four constituent elements takes place upon the equator of two successive maturation spindles. On the first spindle each ring is separated into two dumb-bell figures. On the second spindle each dumb-bell divides into its two simple chromosomes. Both of these divisions are effected by a breaking of the linin fibres between the chromosomes, not by division of the chromosomes themselves.

Toyama's description of the origin of the "Nebenkern" from the remains of the interzonal filaments of the last spermatocyte division is essentially the same as I have given for Caloptenus, but he has followed its fate in the spermatozoön farther than I was able to do. His "mitosoma" may be identical with the body which I find in the neck of the spermatozoön of Caloptenus.

January 19, 1895.

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EXPLANATION OF PLATES.

Figures 1–104 (Plates I. and II.) are from *Cicada tibicen;* Figures 105–244 (Plates III.–V.) from *Caloptenus femur-rubrum.* All figures were drawn by means of an Abbé camera lucida. The tube was drawn, giving a length of 199 mm. Zeiss lenses were used in all cases. Figures 14 and 108 were magnified 90 diameters; Figures 7, 8, 12, 13, and 19–22, 870 diameters; Figures 227–229, 231, and 242, 1,080 diameters; Figures 62–77, 1,400 diameters; all other Figures, 680 diameters.

PLATE I.

Cicada tibicen.

Fig. 1 *a–e*. Spermatids in various stages of metamorphosis.
Fig. 2. Spermatid with nucleus Nebenkörper and small red body.
Fig. 3. Spermatid, tail undeveloped.
Fig. 4. Spermatid immediately after second division of spermatocytes.
Figs. 5, 6. Spermatids.
Fig. 7. Spermatogonium with centrosomes near nucleus.
Fig. 8. Spermatogonium after amitotic nuclear division. Degeneration.
Figs. 9, 10. Spermatids.
Fig. 11. Spermatogonia.
Figs. 12, 13, 21, 22. Stages in development of giant spermatozoa.
Fig. 14. Longitudinal section of testicular follicle.
Figs. 15–18, 24–43. Stages in the metamorphosis of spermatids. See also Figs. 44, 45 (Plate II.).
Fig. 23. Part of compartment *c* of Figure 14. To the left of the connective tissue dissepiment are spermatogonia; to the right, below, are spermatocytes of the first order; above, spermatids.
Figs. 19, 20. Degenerating spermatogonia. Fig. 19 with two extranuclear bodies, centrosomes ?

[illegible] SPERMATOGENESIS PLATE I

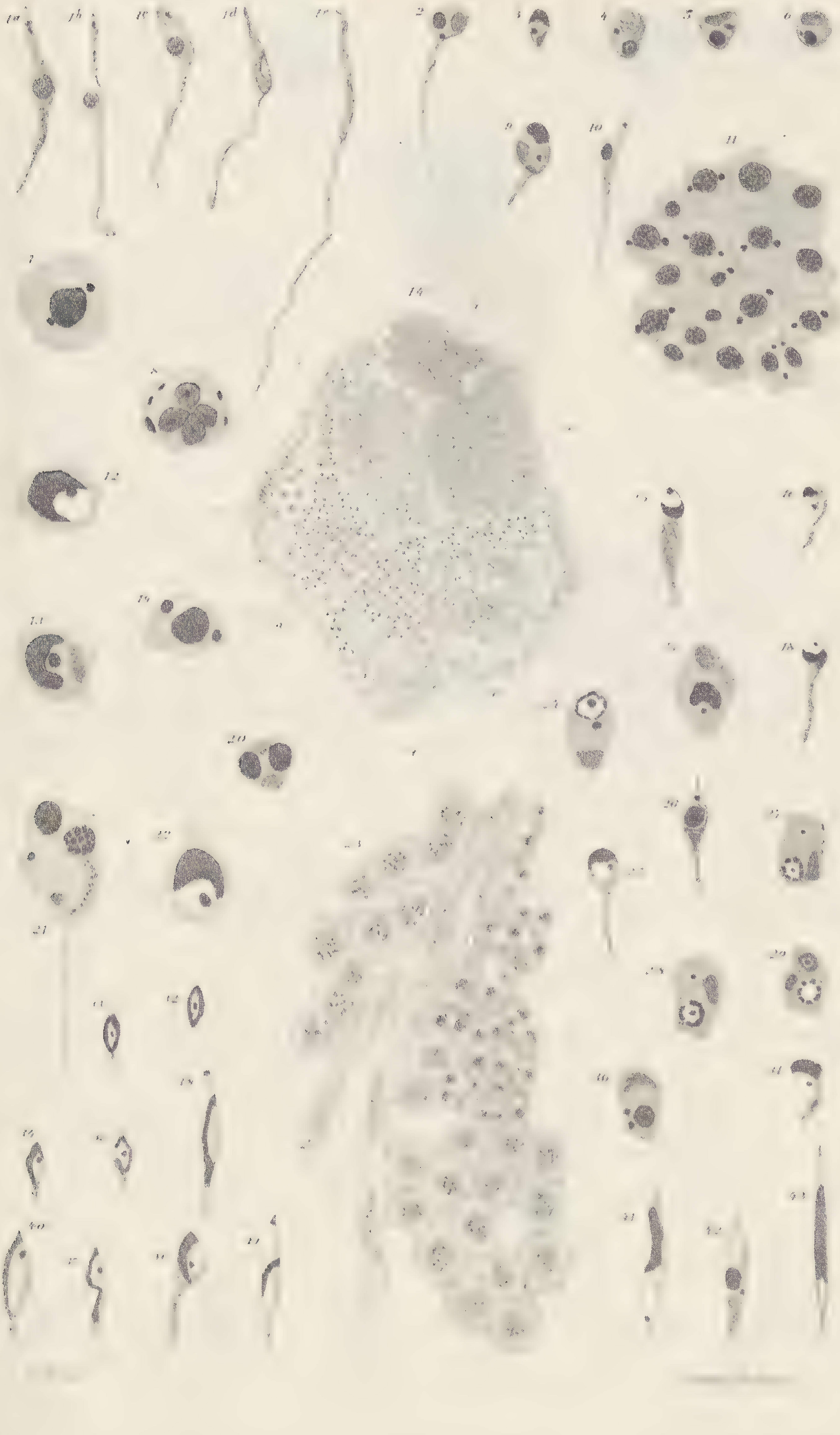

PLATE II.

Cicada tibicen.

Figs. 44, 45.	Advanced stages in the metamorphosis of spermatids.
Figs. 46–48.	Spermatogonia.
Figs. 49–61.	Spermatocytes, showing different positions of the body I suppose to be the nucleolus.
Figs. 62-77.	Metamorphosis of spermatids, × 1,400.
Figs. 78–103.	Metamorphosis of giant spermatids, × 680.
Fig. 104.	Normal spermatids from same microscopic field as Figures 78, 103, × 680.

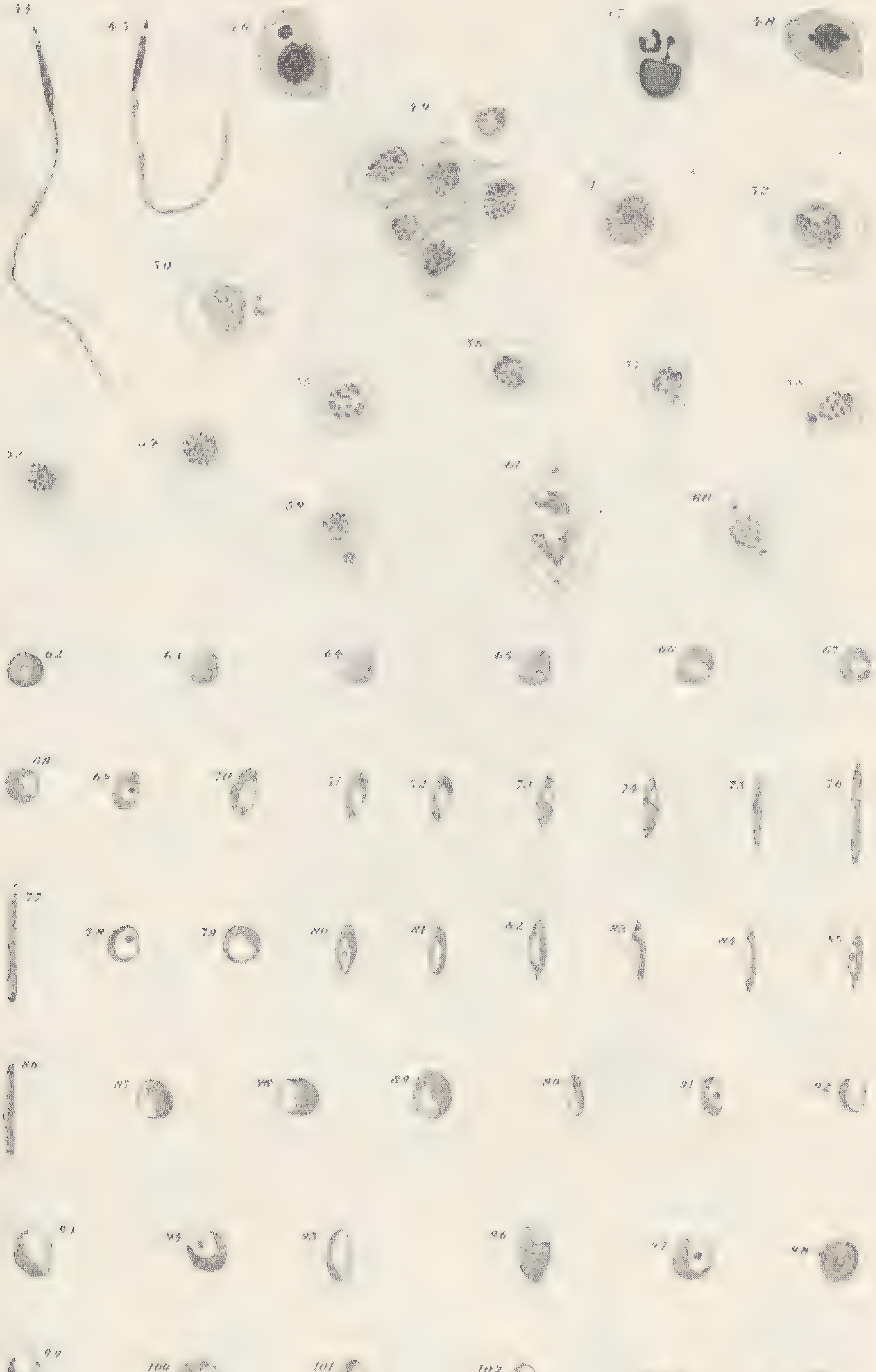
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PLATE III.

Caloptenus femur-rubrum.

Figs. 105–107. Spermatogonia.

Fig. 108. Longitudinal section of testicular follicle. *a*, spermatogonia; *b*, growth zone of spermatocytes; *c*, first division of spermatocytes; *d*, first stages of spermatids; *e*, later stages of spermatids; *f*, abortive spermatogonia.

Fig. 109. Cross-section of vas deferens containing spermatozoa.

Figs. 110, 111. Spermatocytes in the spirem stage.

Figs. 112, 113, 117, 118, 122, 123. Spermatocytes in the first division.

Fig. 114. Spermatocyte of the first order before dumb-bell stage of chromatin.

Figs. 115, 116. Spermatocytes of the first order in the dumb-bell stage of chromatin.

Figs. 119–121, 124. Spermatogonia in division.

Figs. 125, 126, 129. Spermatids just after division of the spermatocytes of the second order.

Fig. 127. Spermatocyte, first division.

Fig. 128. Spermatocyte, second division.

Fig. 130. Spermatocyte before first division.

Fig. 131. Spermatogonium, polar view of spindle.

Figs 132–138. Spermatogonia in division.

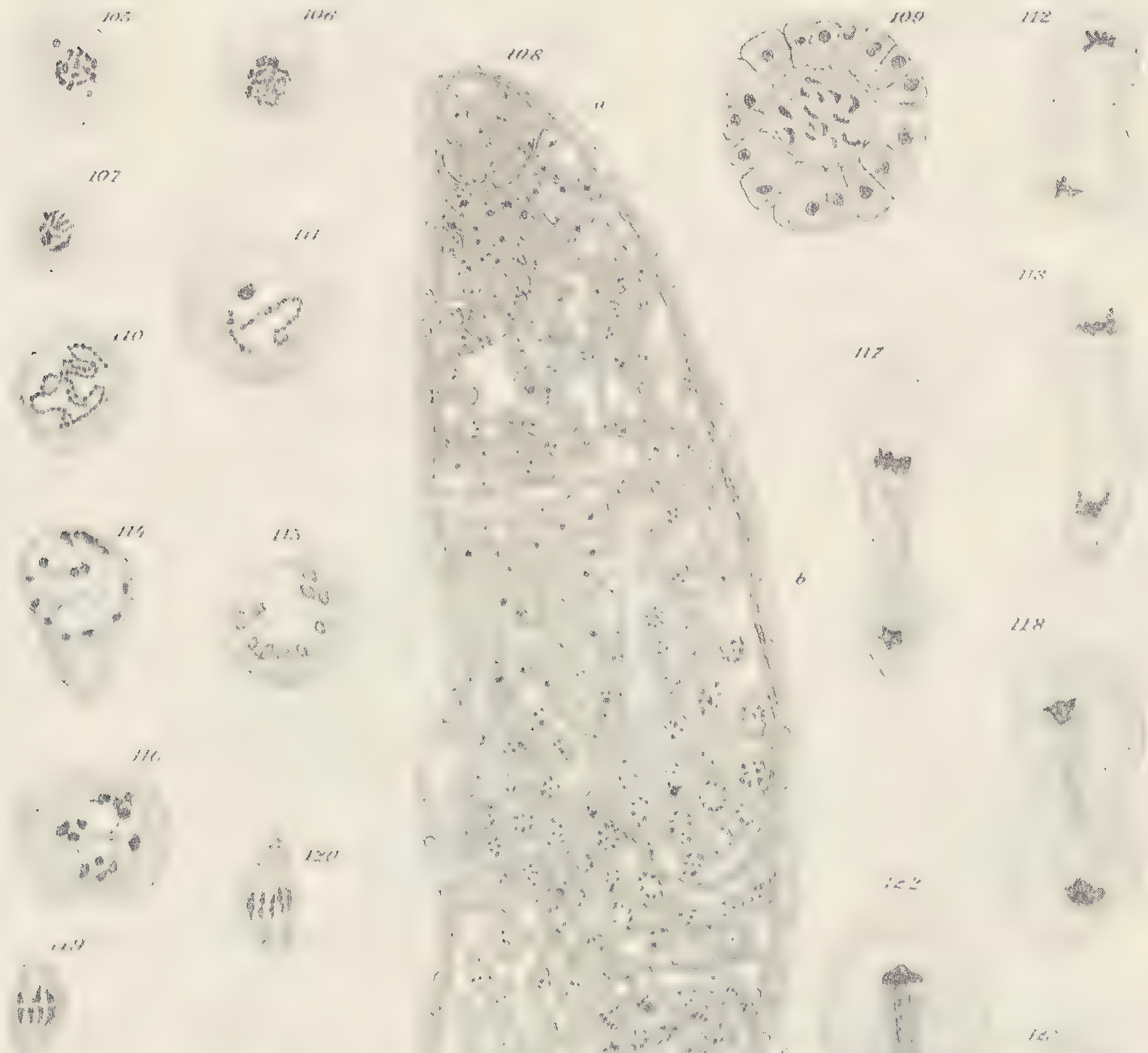
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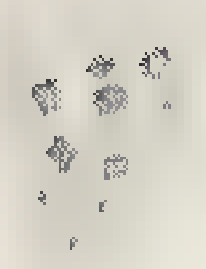
PLATE IV.

Caloptenus femur-rubrum.

Figs. 139–158.	Spermatid metamorphosis.
Figs. 159, 160.	Spermatocytes, first division.
Fig. 161.	Spermatocyte, preparatory to first division.
Figs. 162, 163.	Spermatocytes with chromatin in dumb-bell stage.
Figs. 164–171.	Spermatogonia.
Figs. 172–176.	Spermatocytes, stages in ring formation.
Fig. 177.	Spermatocyte, first division. One centrosome is cut away in another section.
Figs. 178–183.	Spermatocytes, ring stages.
Figs. 184–188.	Spermatocytes, spirem stages.
Fig. 189.	Spermatogonium, tripolar division.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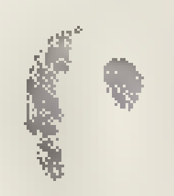
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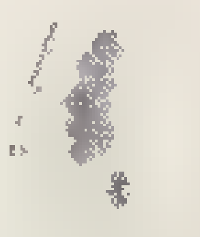
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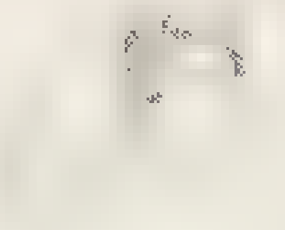
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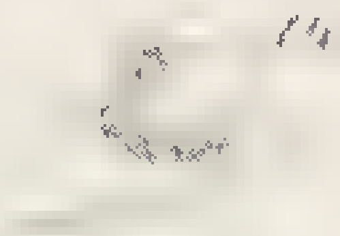
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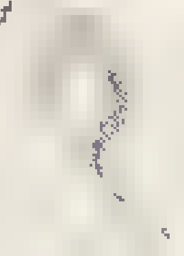
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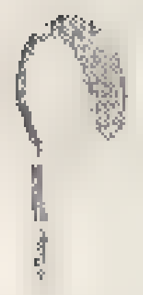
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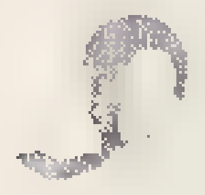
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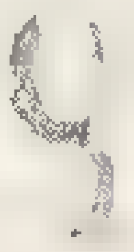
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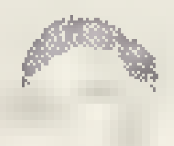
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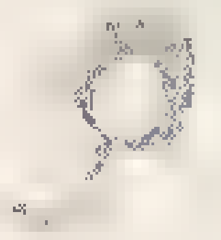
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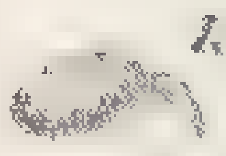
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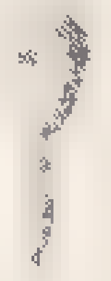
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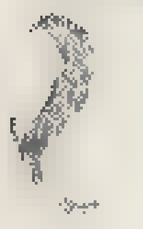
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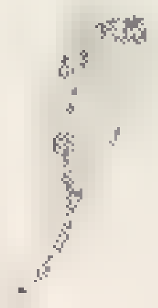
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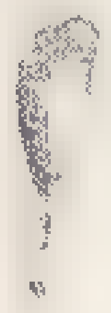
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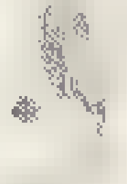
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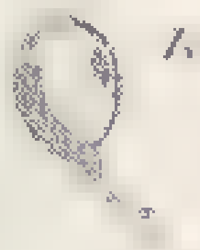
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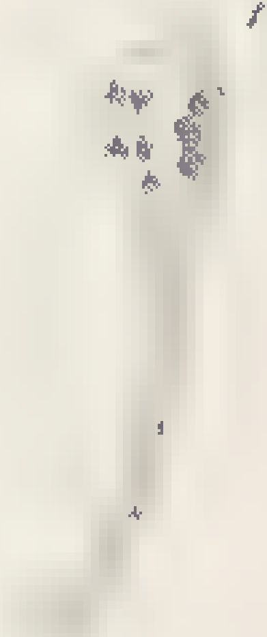
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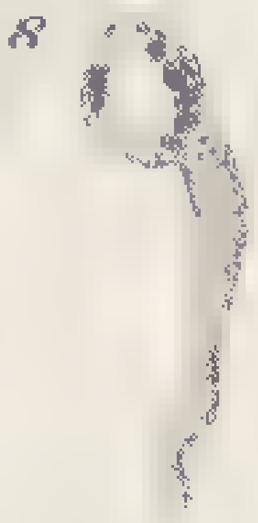
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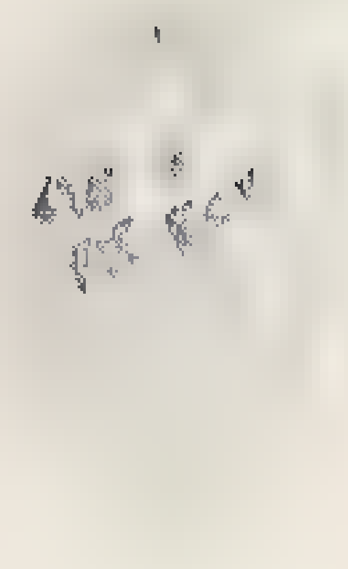
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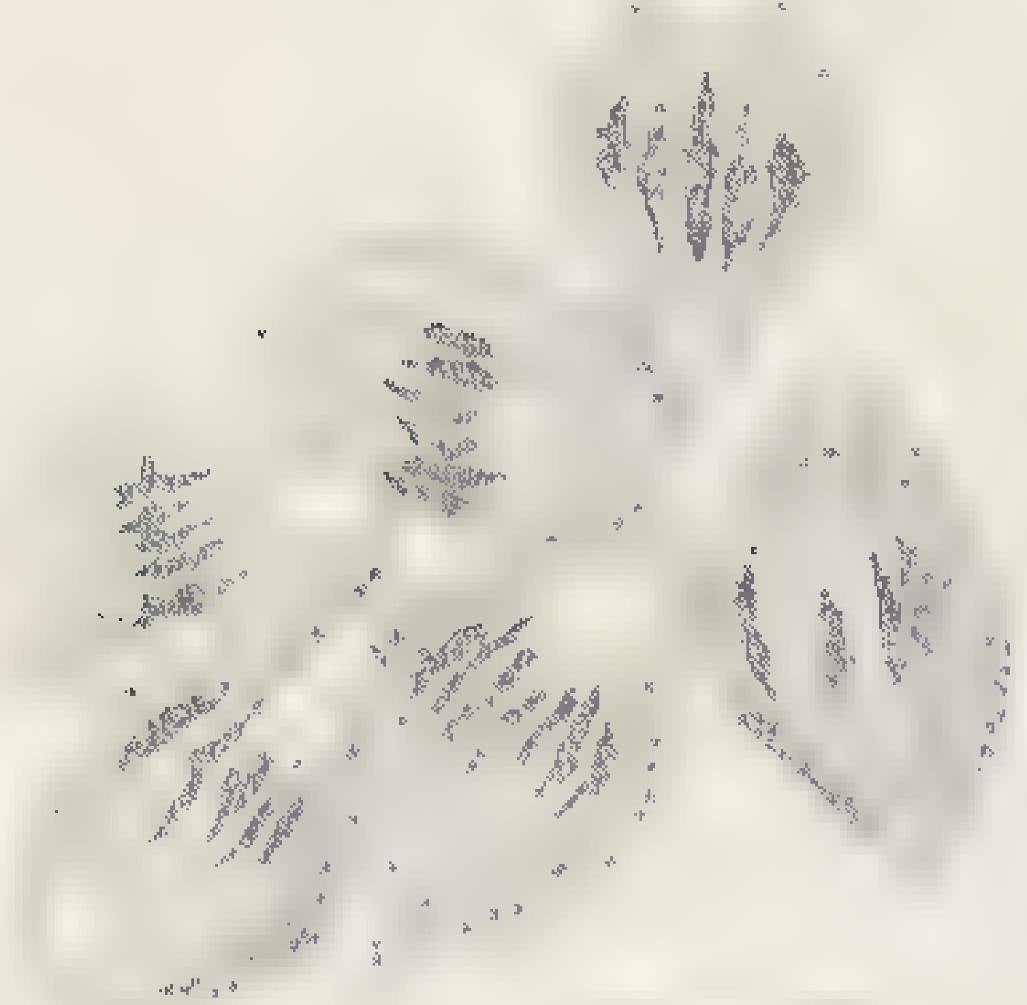
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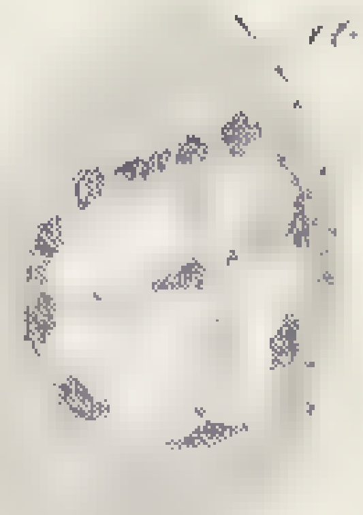
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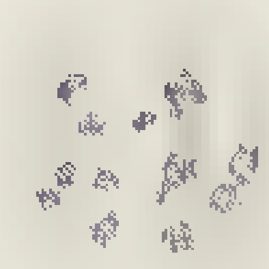
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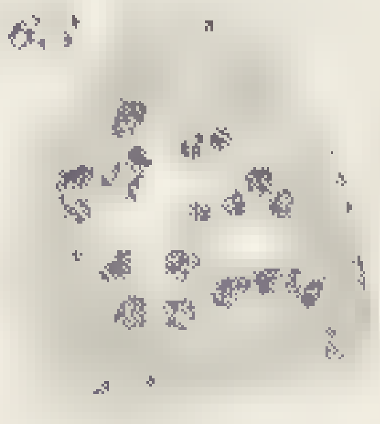
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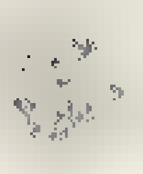
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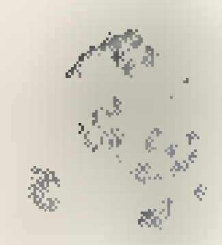
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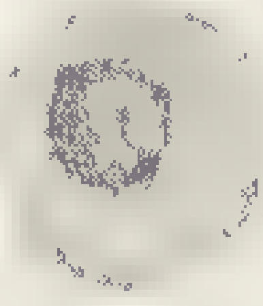
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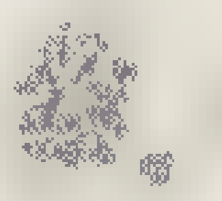
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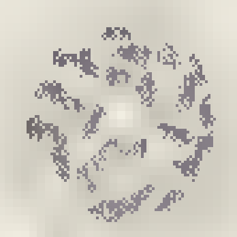
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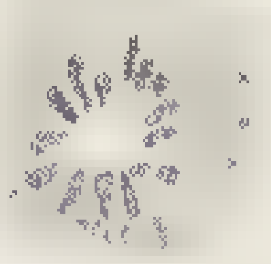
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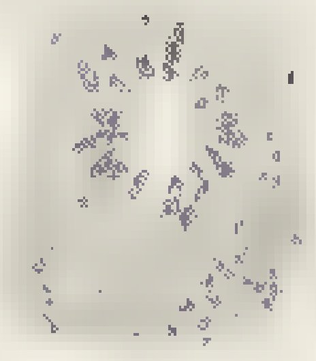
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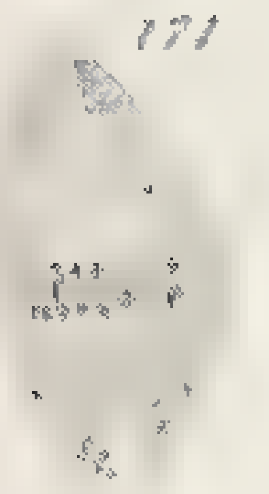
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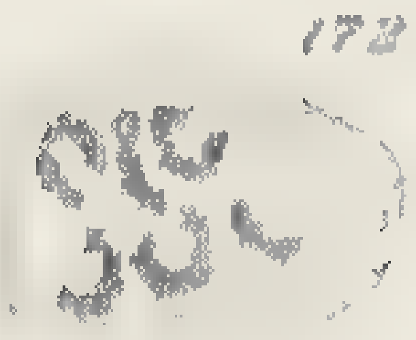
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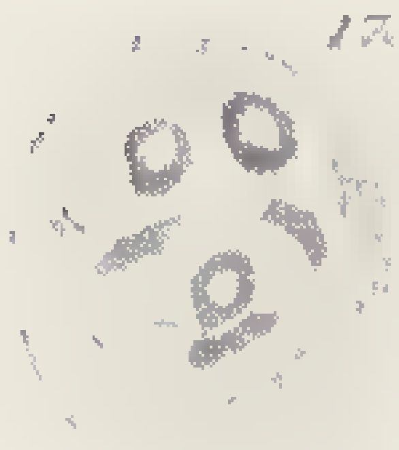
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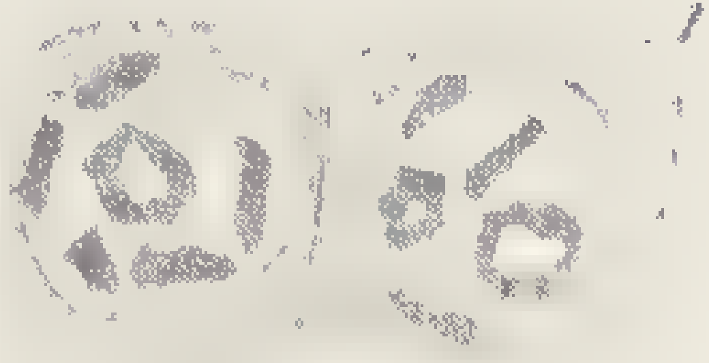
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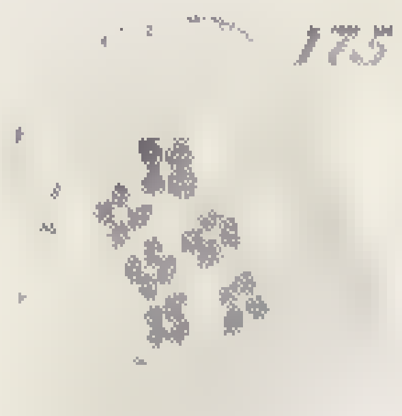
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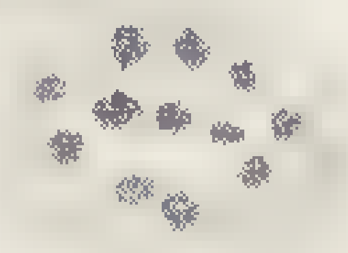
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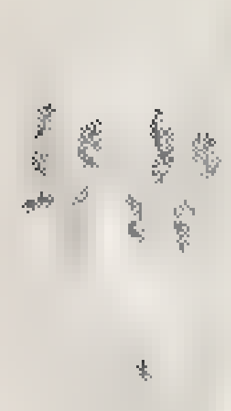
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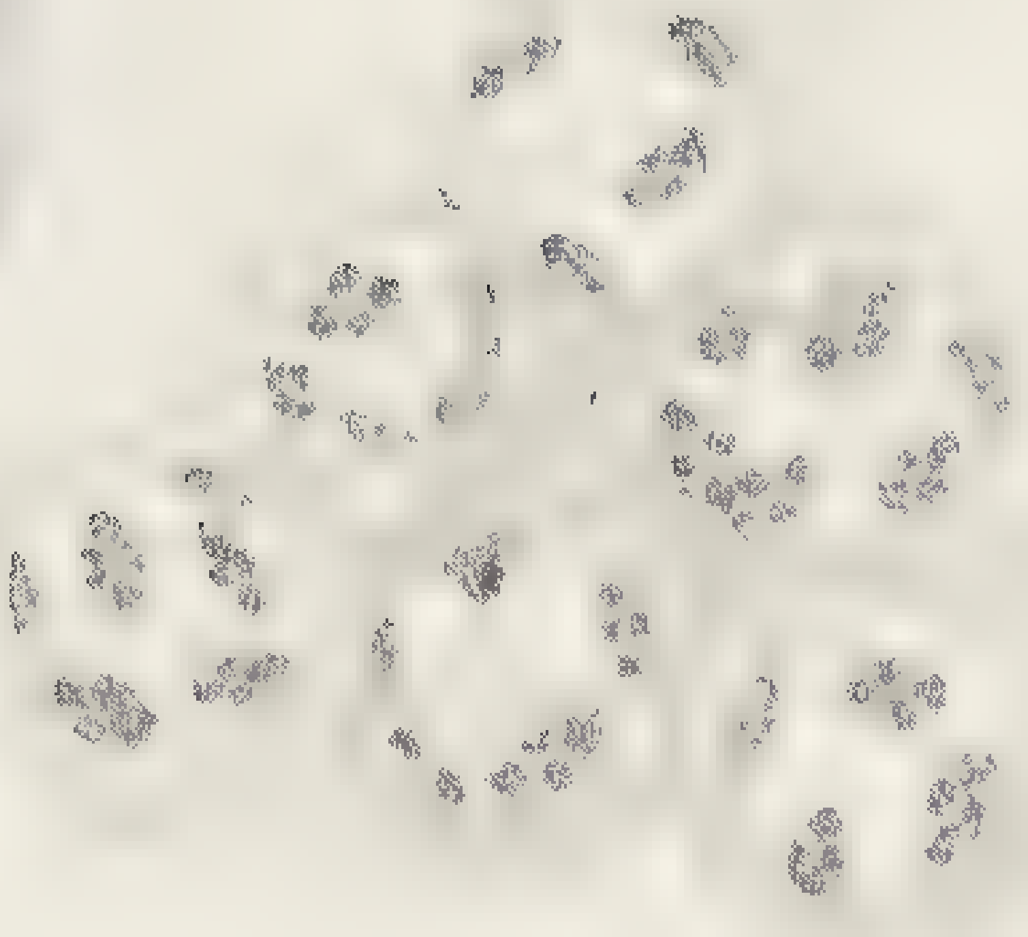
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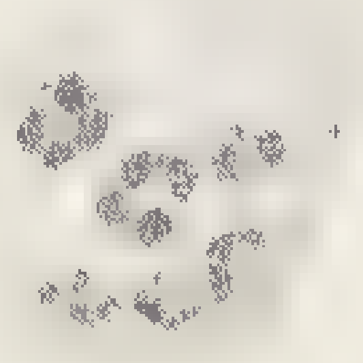
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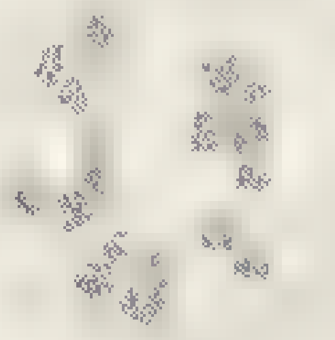
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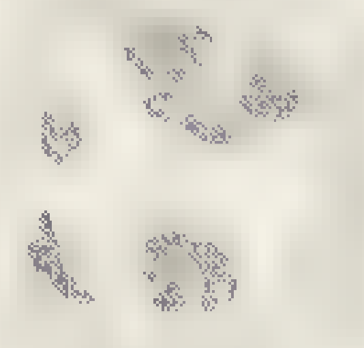
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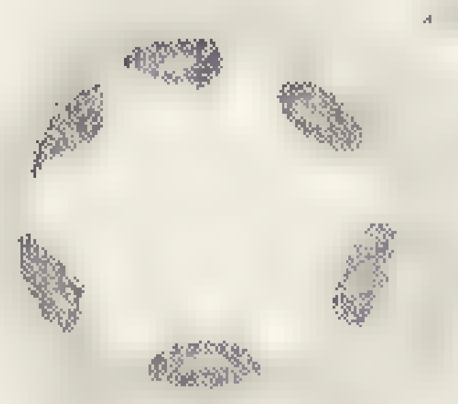
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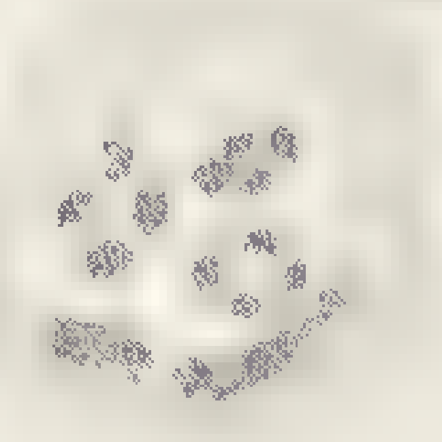
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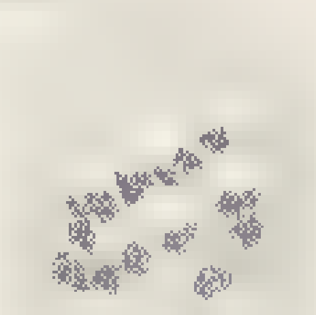
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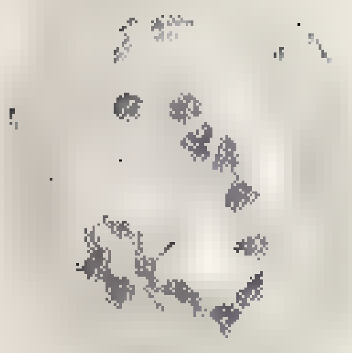
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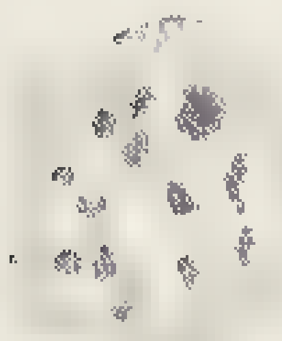
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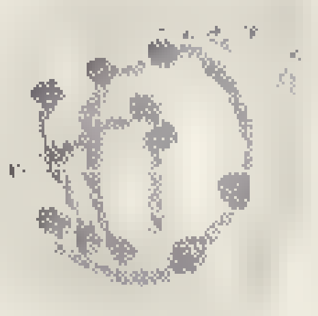
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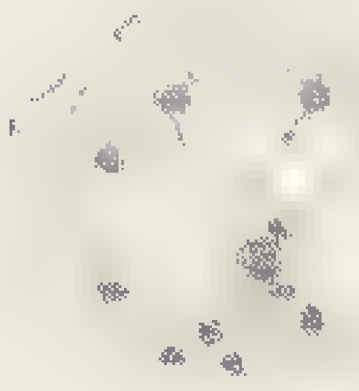
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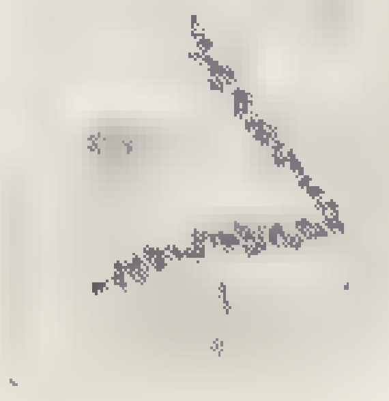
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PLATE V.

Caloptenus femur-rubrum.

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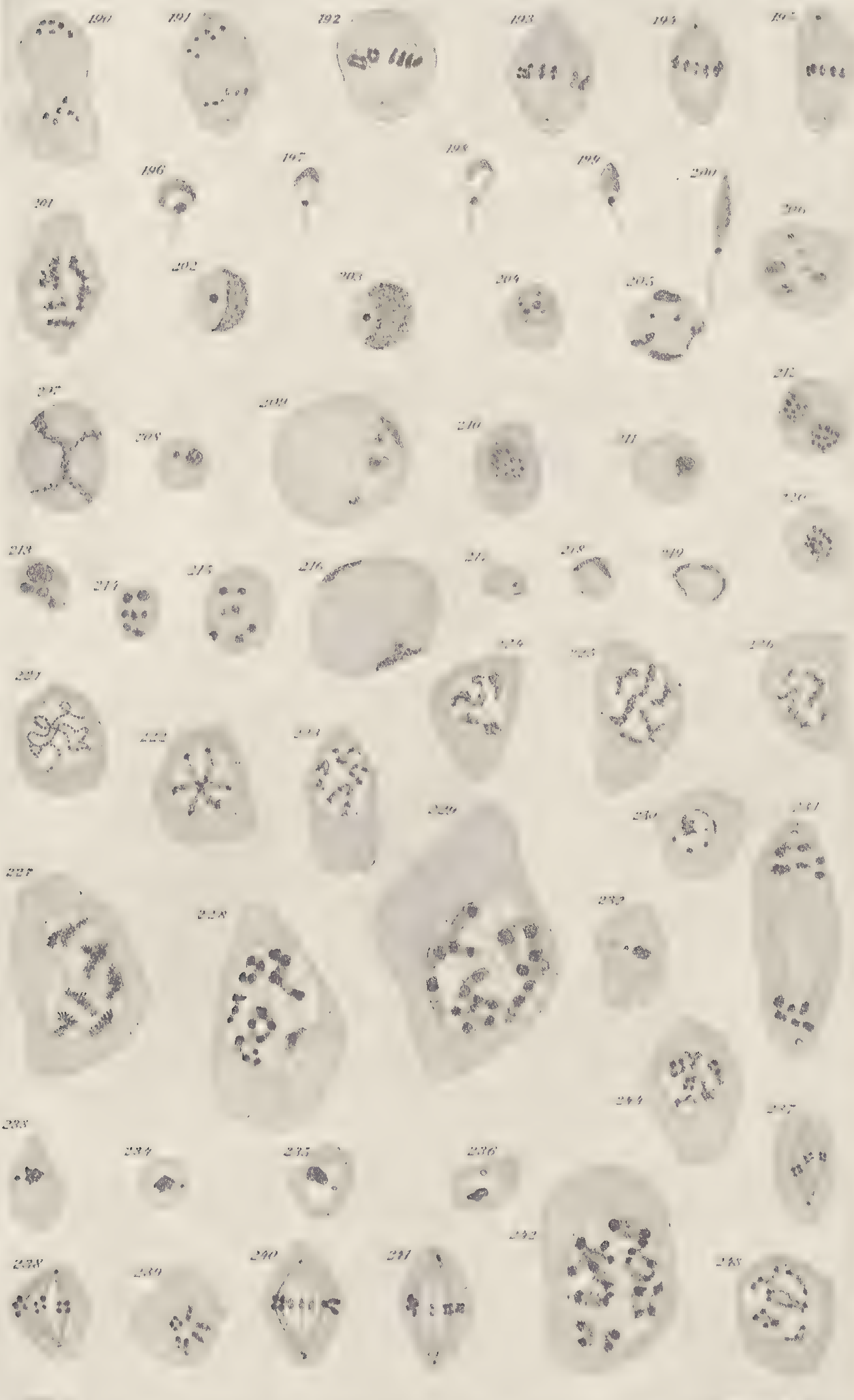
Figs. 192–195.	Spermatocytes, first division.
Figs. 196–200.	Spermatid metamorphosis.
Fig. 201.	Spermatocyte just before ring condition.
Figs. 202–220.	Degenerating cells.
Figs. 221–229, 244.	Spermatocytes, preparatory to first division.
Figs. 230, 232–236.	Spermatids.
Fig. 231.	Spermatocyte, second division.
Figs. 237–241.	Spermatocytes, first division.
Figs. 242, 243.	Spermatocytes, ring formation.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ON THE EARLY DEVELOPMENT OF LIMAX.

BY C. A. KOFOID.

WITH EIGHT PLATES.

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I. INTRODUCTION.

THE question of the origin and history of the mesoderm, and its relation to the body cavity in the Mollusca, is one of prime interest and importance. The employment of the mollusk as the type of the "Pseudocoels" by the Hertwigs ('81) in their "Coelomtheorie" was founded on the non-existence in mollusks of a true body cavity, the mesenchymatous nature of the musculature, and the origin of the nervous system, in part at least, from the mesoderm; in a word, on the nature of the middle germ layer in its origin and later history. Since the publication of this important work many additions have been made to our knowledge of the Mollusca. There is a notable agreement among later investigators, especially Schmidt ('90), Miss Henchman ('91), and Erlanger ('91), as to the *ectodermal* origin of the nervous system in this group. Studies in comparative anatomy, particularly of that primitive group, the Solenogastres, have led to the general acceptance of the view that the pericardium of the Mollusca is the homologue of the cœlom of the "Enterocoels" of the Hertwigs. This view is based upon the relationship of the pericardium to both the sexual and excretory systems, embryology however having lent little support to the view until the recent important work of R. von Erlanger ('91) upon Paludina, in which he demonstrates that the mesoderm originates by a pair of ventral out-pocketings, and that there is a close connection, though not *continuity*, of the paired fundaments of the pericardium with these out-pocketings. As yet his results are isolated, and the hope that a similar origin of the mesoderm might be found in other Mollusca is unfulfilled, at least so far as other investigators are concerned.

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. XLVIII.

The generally vague and often contradictory results obtained by various investigators of molluscan embryology concerning the origin of the mesoderm have made very apparent the need of careful and detailed work along the lines laid down by Rabl ('79), Blochmann ('81), Wilson ('92), Conklin ('92), and Heymons ('93). It is only in the light of such work as this that a classification of sweeping import, like that of the Hertwigs' "Coelomtheorie," can find its final justification, if it has one.

It was with a view of adding something to our knowledge of the details of this subject that the work in hand was undertaken. The pursuit of this has led me unavoidably into the study of the cleavage, and to a certain extent into the field of cell lineage.

Limax seemed for many reasons to be a desirable object for my investigation. The adults are readily procurable, and an abundant supply of eggs whose age is approximately known can be obtained from animals kept in confinement. The absence of a large amount of nutritive yolk leaves the eggs sufficiently translucent for examination *in toto*, and makes section-cutting feasible, though the smallness of the eggs renders their orientation in certain stages difficult.

My work was begun in the fall of 1892, at the suggestion of Dr. E. L. Mark, to whose kindly interest and guidance I owe very much. Agriolimax agrestis L. was the species chosen for the work, as the adults are abundant at that time of the year about piles of rubbish and stone heaps, — in fact, wherever decaying vegetable matter and moisture afford food and a suitable retreat. After the last of November, a supply of adults can generally be secured in greenhouses.

The eggs of other Limaces can also be collected in the same localities, and as those of Agriolimax agrestis are not readily distinguished from some of them, recourse was had to eggs of known parentage only.

II. METHODS.

The most successful method of keeping the animals in captivity was found to be as follows. A tin box with proper ventilation is filled to the depth of one inch with clean sand, which forms a suitable substratum for the retention of moisture. On this is laid down a sheet of moss, to whose under surface the earth still adheres. The leaves of the common plantain furnish acceptable food, and, when this is no longer available, fresh cabbage leaves and apple parings can be used. The eggs, which seem more often to be laid at night, are found in clusters in the soil, or cunningly packed away in the moss itself. The rate of development is such at the ordinary temperature of the laboratory that the eggs collected in the morning will generally be found to have already reached the early stages of cleavage, while gastrulation progresses during the second day, and is completed early in the third. During the first week of captivity the slugs furnish eggs in great abundance; but after that time the number diminishes and the quality deteriorates so rapidly that it is imperative that a new colony be secured. Abnormalities in the living egg show themselves in the early stages by a loose assemblage of the cells, and the increasing opacity of the embryo.

Before hardening the embryo, it is necessary to free it from the envelopes and albumen which surround it. As the eggs of Agriolimax agrestis are much smaller than those of Limax maximus, it was not possible to employ the method described by Miss Henchman ('91) for shelling the eggs. But by inserting two fine cambric needles in one holder, so that the distance between the points is less than the diameter of the unshelled egg, it is possible to hold the egg between these two needles and pierce it by a third. A quick shear-like cut with the third needle against one of the other two tears open one side of the egg and allows the albumen and the ovum to escape from the envelopes. It is very desirable not to entangle the embryo in the viscous matter between the inner and outer envelope, for it is almost impossible to remove this when it is once attached to the embryo. The albumen interferes with section-cutting and obscures whole preparations, so that it is necessary to remove it entirely. This for a long time presented a most serious obstacle to my work. Washing off the albumen with water is a very slow and tedious process, and not always successful. Some of the eggs, after treatment with Merkel's or Flemming's fluid for a short time, were washed with hypochlorite of soda to rid them of the albumen. The difficulty of using this lies in the necessity of stopping the action of the hypochlorite before it attacks the ovum. It frees the eggs of the albumen, and does not interfere with staining, but the proportion of eggs destroyed in the process is very great. When the eggs are thrown into weak chromic acid (one fourth to one third per cent) or weak Merkel's fluid, the albumen is coagulated, and if the exposure is properly timed, the egg can sometimes be freed from its envelopes. It is difficult to get good series of sections of eggs hardened in their envelopes, or but partially freed from them. The process of dehydrating and embedding renders the albumen so hard and brittle that it breaks into bits when a microtome knife strikes it, and generally the whole section becomes shattered. This is especially true of eggs killed in any of the chromic fluids. The method of Schmidt ('90) for the later stages of Limax was employed with success for the early stages. The eggs are thrown into a saturated aqueous solution of corrosive sublimate, and as soon as they have become opaque they are washed in water, shelled, and freed from the coagulated albumen by a gentle stream of water from a pipette. There is less danger of distorting or destroying the egg in the process of shelling by this method than by any other I have employed; the disadvantages are that one is limited to this single killing reagent, and that it is often difficult to remove all the albumen.

The method which gives by far the best results is as follows. The living eggs are placed in normal salt solution (0.75 per cent), in which they are at once shelled, and then freed from the albumen by washing them in the salt solution, which is dropped upon them from a pipette. The operation is carried on in large glass dishes, resembling watch-glasses, but provided with flat polished bottoms, which are placed upon a black tile; this renders the eggs visible to the naked eye. The salt solution dissolves away the albumen, leaving the egg entirely free. It can then be transferred to any desired killing reagent by the use of a capillary glass tube. It is advisable to shorten the exposure in the salt solution as much as possible, for nuclear conditions are somewhat altered by its action. Eggs which have lain in it for ten minutes have their nuclear membranes much distended, and the chromatin, gathered into a homogeneous mass at the centre of the nucleus, surrounded by a clear region of nuclear sap. It is possible, however, by this method to obtain eggs whose nuclear conditions do not seem to be in the least changed.

For killing reagents the following were used: picro-sulphuric; picro-sulphuric with a few drops of one per cent osmic added to it (Erlanger, '91); Perenyi, followed by five per cent alum water; Whitman's Merkel; Fol's modification of Flemming's chrom-osmic-acetic, either alone or followed by Whitman's Merkel. By far the most satisfactory results were obtained by subjecting the eggs to the action of Fol's modification of Flemming's mixture for one minute and transferring them at once to Orth's picro-carminate of lithium. The eggs were allowed to remain in the stain twelve to twenty-four hours, and were then decolorized with acidulated alcohol until the cytoplasm retained but a slight tinge of red. Rapid decolorization with ninety per cent alcohol plus five per cent hydrochloric acid gave very good results. The eggs when properly decolorized have cell boundaries and nuclear membranes sharply marked, and the chromatic elements of the nucleus remain a deep red. Asters show plainly, but centrosomes are not stained. Eggs killed in Flemming's fluid and afterwards bleached by chlorine, or those killed in Merkel's fluid, are satisfactorily stained in Mayer's HCl-carmine. These also must be thoroughly decolorized. Eggs killed in corrosive sublimate were stained in alum-carmine or Czokor's cochineal, but the best results after this killing agent were obtained by the addition of a drop of Delafeld's hæmatoxylin to slightly acidulated water in which the eggs had been placed after hardening in alcohol (Conklin, '92). This is especially valuable for the demonstration of astrocœls in the early stages of cleavage. Satisfactory results were not obtained on whole preparations with Heidenhain's iron-hæmatoxylin or Henneguy's method with permanganate of potash and safranin. The first, however, gives very good results with sections.

The processes of killing, hardening, staining, and clearing were carried on in watch-glasses. Capillary glass tubing was found to be very convenient for transferring individual eggs when such transfer was necessary. Turpentine, xylol, or cedar oil was used as a clearing agent. Eggs can be kept without harm for a long time in turpentine evaporated down to a waxy consistency, or in xylol to which soft parafine has been added. If the xylol is allowed to evaporate, it leaves the eggs embedded in the soft parafine, which can be redissolved by fresh xylol without harm even to these very delicate objects.

The eggs were studied in the clearing agent under a cover-glass placed on glass rollers made of bits of capillary tubing. This allows the use of high-power objectives and the orientation of the embryo in any desired position for a camera drawing. When permanent preparations were desired, they were mounted in xylol-balsam or a solution of dammar in cedar oil. By the addition of a drop of xylol to the margin of the cover-glass, the mounting medium is sufficiently softened to allow the cover-glass to be moved upon the rollers, and the egg oriented as desired, even after the slide has stood for months.

The method of embedding and orienting preparatory to it described by Dr. Woodworth ('93) proved to be very valuable. The ordinary method of orienting in melted parafine on the warm stage with the aid of a lens was also employed. Sections were cut 6.67 μ in thickness, and reconstructions of many stages were made in wax on a scale of three hundred diameters. Transverse, sagittal, and frontal sections were cut; though it was not always possible to orient the embryo exactly, the reconstructions revealed the direction of the sections in cases where there was doubt. Sagittal sections are more readily interpreted than the others, for in them the cells of the different germ layers are shown in the same section in such relations as to be more easily recognized than in sections in other planes.

In the discussion of sections the following orientation is used. The end called anterior is the one toward which the growing invagination is directed. At the time of gastrulation it is the larger end of the embryo. The opposite end is the posterior, and is marked at the stage preceding gastrulation by a greater thickness than the anterior end, due to the presence of the mesoderm. In the early stages of gastrulation the broader and shallower end of the blastopore lies anterior. At the completion of gastrulation the contracted remnant of the blastopore occupies a terminal position at the posterior pole. The chief axis is the antero-posterior one. The ventral surface is marked in the blastula by a greater convexity than the dorsal, but during the period of gastrulation by the growing invagination. Sections are called sagittal that are parallel to the plane which coincides with both chief and dorso-ventral axes; frontal, those that are perpendicular to the dorso-ventral axis; transverse, those that are perpendicular to the chief axis.

III. NOMENCLATURE OF SPIRAL CLEAVAGE.

The earliest full discussion of spiral cleavage occurs in Blochmann's admirable work upon Neritina ('81). Fol ('75 and '76) had described the early stages in the cleavage of the Pteropods and the Heteropods, and Rabl ('79) the cleavage of Planorbis; but neither had entered into a full discussion of the lineage of the cells or the spiral character of the cleavages with which he was dealing. In Neritina the cleavage is unequal, and at the formation of the first set of micromeres we have the appearance of a small protoplasmic mass budded off from a larger mass. This conception of the cell division — the derivation of a small part from a large part — dominated Blochmann's nomenclature both of cells and of spirals. Accordingly, we find him designating a large mass of protoplasm, both before and after the small mass is budded off from it, *by the same name.* So also, when he comes to compare the spiral with the motion of the hands of a clock, he regards the *small* cell as *moving away from the large* cell, and designates the spiral accordingly. Other investigators of spiral cleavage — Lang ('85), Conklin ('91 and '92), Wilson ('92), Heymons ('93), Lillie ('93) — have, like Blochmann, dealt with forms presenting a greater or a less inequality in cleavage, and have found it convenient to employ the system inaugurated by Blochmann for their nomenclature of cells and spirals. There has arisen in the usages of these various authors, however, considerable confusion in the detailed application of their nomenclatures to this basis of reference. Indeed, as I have pointed out in a previous paper (Kofoid, '94), an author is not always able to avoid inconsistencies. This state of affairs is confusing and extremely annoying to the student who wishes to make a comparative study of cell lineage. However much the introduction of a new system of nomenclature is to be deplored, it seems to be justified for the following reasons. Cell lineage deals primarily with the *descent and fate of cells*, and is most conveniently traced by following the history of their *nuclei;* it is only secondarily concerned with the amount of yolk or protoplasm in the cells. The founding of a system of nomenclature, therefore, upon the relative sizes of cells, ignores wholly this fundamental proposition, and substitutes a basis of varying and uncertain nature. Furthermore, this system has caused the introduction, perhaps not necessarily, of the custom of designating cells of different generations by identical names; thus *A* may be a cell of any one of a half-dozen different generations. In this, too, the principle of descent is ignored.

Finally and principally, the basis hitherto employed affords *no solid ground whatever for comparisons*, for it gives no logical method to be employed in cases of *equal* cleavage; and its application must vary with the varying distribution of the large cells in different species of animals. Thus it comes about that "homologous" cells, i. e. those of identical descent, must according to this system be named differently in different animals. It may be that the system as applied by these authors does furnish a means, readily grasped by the eye and the mind, of following the lineage in the particular form studied; but so long as it fails to form a basis for comparison, it is open to serious objection. It was with a view of meeting this objection and suggesting a suitable basis of comparison that I proposed, in a paper ('94) to which I must refer the reader for a detailed description, an entirely new principle of nomenclature of both cells and spirals. This is a strictly genealogical system, giving to each cell in the line of descent a separate designation, one determined moreover by the constant spatial relations common to all eggs, and not by the inequality of the cleavage characteristic of individual species.

The system presupposes the division of the egg into four quadrants designated *a, b, c, d*, placed in the order in which the hands of a clock move. These quadrants are occupied by the four blastomeres, *the quartet*, of the third generation. When this quartet, or any other quartet of the later stages, divides, forming two quartets, each cell is designated as follows: (1) by a letter indicating the *quadrant*, as, e. g., a; (2) by a first exponent indicating the *generation*, a^3, a^4, etc.; (3) by a second exponent indicating the *position* of the quartet with reference to all other quartets of the same generation, potential or actual, the quartets being numbered from the vegetative toward the animal pole, as $a^{4.1}$, $a^{4.2}$, etc. Thus the cell a^3 divides, forming $a^{4.1}$ and $a^{4.2}$; in the second exponent the *odd* one being always given to the cells of the quartet which is nearer the vegetative pole, and the *even* to those of the quartet nearer the animal pole. I have previously described ('94) the simple and constant manner in which the designation of the daughter cells can in every case be derived from that of the mother cell.

It may be well to call attention here to the significance of this system of nomenclature. It designates cells as they might be named in the simplest possible mathematical and mechanical conditions of a cleaving egg, i. e. equal, regular cleavage pervading all the cells of a generation at the same time. In such a case we should have all the quartets of a generation actually present and numbered in the regular succession of their position from the vegetative to the animal pole. The possibility of referring all forms of spiral cleavage to such a simple type is obvious, and the advantage, if not indeed the necessity, of such a reference as a basis of comparison is equally apparent. The fact that in the application of this system the exponents have little or no significance, or are even misleading, as to the *actual* number of quartets present between a given quartet and the vegetative pole, is thus no obstacle, when once the real significance of the system is understood. In fact, it is rather an advantage that the regions of most rapid growth in the embryo are thus prominently designated. There are doubtless objections that will be encountered in the application of the system. After the ninth generation of cells the exponents become exceedingly cumbersome, but this is an objection which applies to all other systems as much as, or to even a greater extent than, to this; and it may perhaps in many cases be avoided in the later stages by the introduction, for teloblasts and their progeny, of subordinate dichotomous systems based on combinations of the numerals 1 and 2, as introduced by Chabry ('87), and later adopted by Wilson ('92) in his subordinate systems. It is also true that the system, as proposed, does not optically differentiate the macromeres and the primary, secondary, and tertiary micromeres where it is desirable to distinguish these groups or their immediate descendants. This however is readily accomplished by the use of differential type, or even by other letters of the alphabet than *a*, *b*, *c*, *d*, but used in the same order.

There seems to be no doubt that this system can be applied wherever it is possible to divide the cleaving egg into equivalent quadrants, and thus to distinguish quartets of cells. I have myself applied it to the spiral cleavage described for various forms (see review of the literature), and my friend, Mr. Castle, has applied it successfully to the bilateral cleavage of Ciona and to that of Clavelina as described by Van Beneden et Julin ('84).

To make this system available in all cases, it is only necessary to apply the second exponent in a constant manner with reference to some spatial relations; e. g. in the case of Tunicate cleavage, with reference to the sagittal and transverse planes, starting in all cases from the vegetative pole.

In conclusion, it hardly needs to be suggested that the generation basis of comparison is about the only one that can be employed between the various types of cleavage; and I would add that it promises to be useful in the discussion of precocious development.

IV. GENERAL SKETCH OF THE DEVELOPMENT.

Limax has spiral cleavage of the typical form, the spirals alternating in successive cell generations, right spirals resulting in the even generations and left spirals in the odd. The mesoderm is derived from the left posterior quadrant, and, as in Nereis, Umbrella, Crepidula, and Unio, the first mesoblast cell is $d^{7\,2}$. An ephemeral, recurrent cleavage cavity appears at the two-cell stage, and recurs as late as the completion of the period of gastrulation. This cavity is excretory in function, and is induced by the environment of the egg. The primary mesoblast divides bilaterally, ultimately sinks below the general level, and forms two bilaterally placed mesodermal bands extending anteriorly. Their formation precedes and accompanies gastrulation, no lumina appearing at any time within them. The blastopore is at first broad and shallow, but it gradually deepens at the anterior end, and disappears from the posterior margin anteriorly, forming an elliptical pit on the median ventral surface. By a rapid growth in the latero-anterior lips of this pit, accompanied by an accumulation of mesoderm in these regions and a general readjustment of the axes of the embryo, the opening leading into the archenteron assumes a position at the former posterior margin of the blastopore. This remnant of the blastopore comes to lie in the anal region; the mouth breaks through at a later period upon the ventral surface of the embryo.

V. CLEAVAGE.

Introductory.

The cleavage of the egg of Limax takes place with considerable rapidity. The eggs are generally laid, in captivity, during the night, and in the morning one finds stages from the one-cell to the sixteen- and occasionally the twenty-four-cell stage. By six o'clock in the evening these eggs have reached the stages of twenty-four to forty or more cells. Gastrulation begins during the second day, and is completed on the third day. There is, however, much variation even in a lot of eggs found in one mass, and evidently laid by one individual. These differences may possibly be due to differences in the time of fertilization. Temperature exercises a profound influence on the rate of cleavage. Eggs about to pass into the twenty-four-cell stage at 6 P. M. were placed over night in a temperature a few degrees above freezing, and were found to have just reached that stage at 8.30 the next morning, and, though restored to the temperature of the laboratory, they did not progress to the next cleavage until 2 P. M. There are a few "stages" in the cleavage that are well marked, i. e. periods of apparent inactivity in which the egg continues for some time. These are the the two-, four-, eight-, sixteen-, twenty-four-, forty-four-, and sixty-cell stages. The periods alternating with these are marked by mitotic conditions in all or a part of the cells of the egg.

The animal pole of the mature and undivided egg is marked by the presence of two polar globules. These generally differ in size, the more distant, i. e. the first, being the larger. In stained preparations the larger one often contains a distinct nucleus with nuclear membrane and chromatic granules (Plate III. Figs. 20, 21). In the case figured here the two globules are closely applied to the surface of the egg. In the majority of instances, however, they lie at some distance from the egg in the albumen, and in the living egg often seem to have no connection whatever with the vitelline surface. Thus it happens that the polar globules are removed with the albumen in by far the larger part of the eggs shelled. A phenomenon observed occasionally in the later stages of the living egg is the increase in size of one of the polar globules and its subsequent collapse (Plate I. Figs. 9–11). In one case the globule reached a diameter half that of the egg itself. This is apparently caused by the absorption of fluid from the albumen, and in the case noted was followed by a collapse and a return to the normal size and shape. The eggs of Agriolimax agrestis vary a great deal in size, the limits being from 80 μ to 160 μ in diameter. The average size is about 100–110 μ.

A. Orientation of the Embryo.

In my treatment of the subject the orientation employed by Wilson ('92), Conklin ('92) and Heymons ('93) is followed. The first cleavage plane is transverse, the second sagittal, in relation to the future embryo. The polar globules are dorsal, the macromeres are ventral. This does not, however, distinguish the anterior and posterior poles, and I know of no way in which they can with certainty be determined in Limax. The cells of the two ends are equal in size, generally, and when slight differences can be detected on careful measurement, it is impossible to follow these differences during the protean phases of cleavage that intervene between the two-cell stage and the appearance of the first mesoderm cell, marking the posterior pole. Inasmuch as the mesoderm cell ($d^{7.2}$) comes from the left posterior quadrant, and is itself quite a large cell, while its sister cell ($d^{7.1}$) does not seem to be much smaller than other members of its quartet, I have always placed the larger of the two touching quadrants of the basal quartet in the position *left posterior*, rather than right anterior. I have been compelled to orient arbitrarily in many cases, when no difference in size could be detected, choosing one of the two positions 180° apart. In choosing the larger cell I have not followed the type of Umbrella, where without doubt the mesoderm comes from the smaller of the two cells in contact at the ventral cross furrow.

B. Discussion of Cleavage.

SECOND GENERATION. FIRST CLEAVAGE PLANE. TWO CELLS.

Plate I. Fig. 14.

It should be noted that the phrase "generation of cells" is used in its strict genealogical meaning, and not in the sense in which Fol ('75, '76), Blochmann ('81), McMurrich ('86), and Heymons ('93) have used the phrases "generation of micromeres" and "generation of cells." The processes of maturation, fecundation, and the formation of the first cleavage furrow have been described in detail in Dr. Mark's classic work upon Limax campestris. His published work was not carried beyond this stage, though he had continued his researches far into the later stages of development. The appearance of Fol's work ('80), which dealt largely with the embryology of Limax, and the pressure of other duties, have interfered with the completion and publication of his studies. Dr. Mark has very kindly placed his numerous drawings and careful notes in my hands, and they have been of invaluable assistance to me in my work. I shall not attempt to add to his complete description of the formation of the first cleavage plane, but shall begin my work with the stage represented in Plate I. Fig. 14. This is a lateral view of an egg which has just completed the first division. Warneck ('50) has stated that in Limax and Lymnæus this plane is oblique to the axis of elongation, instead of transverse to it, and has distinctly said that this conclusion was not based upon a deceptive orientation of the egg. Fol ('75) has described a similar occurrence in Cymbulia. I have found no evidence that in the least confirms this statement of Warneck's. At the stage shown in Figure 14 the two nuclei lie close to the approximated surfaces of the blastomeres, at a level about midway between the animal and vegetative poles; they are still quite small, and have only a very delicate membrane. Each has an elongated oval outline, with the long axis extending toward the astrocœl of the cell in which it lies. Their position indicates that in the progress of the cleavage furrow toward the vegetative pole the nuclei (daughter segments) were in some way carried downward toward that pole. Mark ('81) has described such a procedure in the eggs of Limax campestris. There are a number of deeply staining granulations in the peripheral part of the cell adjacent to the nuclei, which probably constitute the remnant of the cell plate; there is thus every indication of recent cell division.

The astrocœls appear as clear areas, almost as large as the nuclei, containing a few scattered deeply staining granules. These clear areas have no limiting membrane; they are, however, devoid of the granular structure of the surrounding protoplasm, and are the centres about which the radiations constituting the asters are arranged.

The position of the astrocœls with reference to the nuclei is worthy of note. They are removed some distance from the nuclei toward the animal pole of the cells in which they lie. A comparison of this figure with that of a later stage shown in Figure 5 indicates that the astrocœls are migrating toward a region where later the nuclei are found. It must seem therefore from the conditions in Figure 14 that the nuclei are preceded in this migration by the astrocœls. This recalls the shifting of male and female pronuclei attributed to the astrocœls by Conklin ('94) in Crepidula.

In the living egg of this stage, when the cells have reached a perfectly spherical shape, each blastomere seems to be entirely independent of the other, and not the least trace of any contact or connecting protoplasm can be detected between them. Each has a definite, unbroken contour, and in most cases there is an appreciable space between them, which shows no differentiation from the surrounding albumen. In the egg shown in Figure 14 the separation is not so great as it apparently is in the living egg. It is an interesting phenomenon, and raises the question as to the existence of any actual protoplasmic connection between the blastomeres in the stage following constriction. It is impossible to answer the question satisfactorily from observation of the living egg, for there is the possibility of the existence of a thin sheet of protoplasm which, on account of its transparency, thinness, and optical resemblance to the surrounding albumen, cannot be detected. The egg shown in Figure 14 was shelled by the process described in the preceding pages, and washed free from the albumen by normal salt solution, transferred in capillary tubes a number of times in the process of preparation, and, after mounting in balsam, was rolled over in various directions repeatedly *without a separation of the two blastomeres.* The two cells have each of them a definite and sharp outline at all planes of focusing, and even under high powers of the microscope no deeply stained granular bridge of protoplasm can be detected between them. It is only by very careful focusing that the rather vague, transparent, unstained connection between the cells can be seen. So far, then, as this preparation goes, it shows that there is a physical band of connection between the two blastomeres in this stage of greatest separation. The nature of this connection is problematical. It may be the Schleimschicht of Warneck ('50), or it may be a continuation of the "differentiated superficial portion of the yolk" noted by Mark ('81), there being no evidence of a well differentiated vitelline membrane. If the latter, then there is physical continuity in the living substance of the two blastomeres, and the appearance in the living egg is deceptive. Experimentation might possibly settle this interesting question.

The two cells now lose their individual spherical contours, owing to their apposition and the mutual flattening of the two faces in contact. Thus the nearly spherical outline of the whole egg is re-established. See Figures 1–6, which form a series showing successive conditions of a single egg, and render a detailed description of the process unnecessary.

The alternation of the rounded and flattened condition of cells during and subsequent to mitosis has been very generally observed in the stages of cleavage, but the explanation of the causes which underlie this phenomenon seems as remote as ever. Whitman ('78), in his discussion of the cleavage of Clepsine, concludes: "The cause of the separation and of the subsequent approach is undoubtedly the nucleus. . . . The proof that this is an electrical phenomenon is at present wanting, but the facts seem to point in this direction very strongly." Our increased knowledge of the part that the cytoplasm plays in the process of cell division, especially the directive rôle of the centrosomes, has suggested another influence to which we may turn for a solution, though the nature and exact operation of that influence is by no means definitely settled.

This first cleavage plane divides the egg into equal or approximately equal cells. In some cases, by careful measurement, a slight difference in size could be detected; in one case, for example, one of the cells measured 19 × 26 units of the ocular micrometer, and the other 20 × 27, when viewed from the animal pole. The theoretical consideration of the orientation of the early stages will be taken up later; suffice it for the present to say that the orientation adopted by Wilson ('92), and later by Conklin ('92) and Heymons ('93), will be employed in the present paper. The first cleavage plane, then, cuts the egg into an anterior half, *A B*, and a posterior half, *C D*, i. e. it is perpendicular to the antero-posterior axis of the egg.

The discussion of the cleavage cavity will also be deferred till a later part of the paper is reached.

THIRD GENERATION. SECOND CLEAVAGE FURROW. FOUR CELLS.

Plate I. Figs. 6-8; Plate II. Fig. 15-18.

The second furrow is formed, at the ordinary temperature of the laboratory, about two hours after the appearance of the first. Like the first, it is preceded by an elongation of both cells in a direction at right angles to the plane of the division. Figure 15 (Plate II.) presents a view from the animal pole of a stage preparatory to this cleavage. The egg here represented is an exceptionally large one, being about 160 μ in its longest diameter. Each cell contains a spindle lying in its long diameter and nearer the animal pole. If the egg be viewed exactly from the animal pole, it is found that two of the asters — the rays of which are made more prominent in the figure than those of the remaining two — lie at a higher level than their mates. The same fact is brought out in a lateral view of this stage (Plate I. Fig. 7). Of the four asters the two having the same level lie in diagonally opposite quadrants of the egg. If we orient the egg so that the first plane of cleavage is transverse, no matter which pair of cells is placed anteriorly, and name the four asters *A*, *B*, *C*, *D*, in the accepted order, beginning at the left anterior quadrant, we shall have the asters *A* and *C* at the higher level, *B* and *D* at the lower. The slight difference in size between the two cells of this egg (Plate II. Fig. 15) has been previously noted. There is also a very slight difference in the stage of mitosis exhibited by the two cells, the larger being slightly more advanced than the smaller. A difference in the time of cleavage of the two cells of this stage has come under my observation in Limax a number of times. It is, however, not prevalent, and it is impossible to correlate it with any difference in the size of the two blastomeres. In Nereis, Umbrella, Cyclas, Unio, and many other forms, there is a well marked difference in size and a correlated difference in the time of division, the *smaller* cell being generally the first to divide.

Figure 8 (Plate I.) represents the second furrow just before its completion. The difference in level noted in the asters here finds its counterpart in the position of the partially formed blastomeres, the order of arrangement being the same as in Figure 15 (Plate II.). The planes of division are perpendicular to the axes of the spindle. They are therefore not continuous, but both are oblique to the vertical axis and in opposite directions. The posterior plane (separating *C* and *D*) passes from above toward the vegetative pole and the right, the anterior (separating *A* and *B*) from above toward the vegetative pole and the left. Inasmuch as the two derivatives do not lie at the same level, we may test the existence of the spiral; viewing the egg from the animal pole, and going from the lower derivative to the upper, we pass in a direction opposite to that in which the hands of a clock move; this oblique position of cognate cells may be referred to as a left spiral. It should be noted that this position of the cells is predetermined by the inclination of the spindles, which exists before there is any constriction of the cytoplasm. As in the case of the first cleavage plane, the constriction progresses most rapidly from the animal pole. Toward the close of the process of constriction the daughter cells are united by only a bridge of protoplasm, which is nearer the vegetative pole. Figure 16 (Plate II.), from a preparation of a slightly older stage, shows a similar bridge of protoplasm, but it is much nearer the animal pole.

The period of constriction is followed by a stage similar to that described for the two blastomeres, in which each of the four blastomeres assumes a spherical contour and stands out sharply and distinctly from its neighbors. This phase soon passes, and within half an hour the egg has assumed the condition of Figure 9 (Plate I.). This is the typical four-cell stage of the spiral type of cleavage, and therefore merits further description. (See diagram of this stage on page 52, Figure A.) The four cells, *A*, *B*, *C*, *D*, occupy the left anterior, right anterior, right posterior, and left posterior quadrants respectively. Each cell presents to the exterior a rounded, convex surface, and upon its inner side has three facets of contact, — the first and third with the cells of the adjacent quadrants, the second with the cell of the diagonally opposite quadrant. This last facet is triangular in shape, with its base at one pole and apex near the centre of the egg. The vertical axis of the egg lies in the planes of these central triangular facets. The bases of the central facets coincide with the well known cross furrows of the animal and vegetative poles of the egg (compare Plate II. Fig. 17). The cross furrow of the animal pole lies between the cells *A* and *C*, and extends from *D* to *B*, that of the vegetative pole lies between *B* and *D*, and extends from *A* to *C*. Thus by this mutual adaptation of the cells to one another, the spheroidal form of the egg as a whole is, in a degree, again restored, and here, as in the two-cell stage, persists during the period of "nuclear quiescence." I have referred to the condition in Limax as "typical." I mean that the conditions are simple, and that the modifying influence due to the presence of a large amount of yolk, and its equal or unequal distribution among the four blastomeres, is absent.

A comparison of the conditions presented here (Plate I. Fig. 9, Plate II. Fig. 17, and Fig. A, p. 52) with the same stage in other animals shows how profound the modifications are. In Limax the dorsal and ventral cross furrows are approximately equal in length, and as seen from the animal pole lie nearly at right angles to each other. In Nereis (Wilson '92) the dorsal furrow is largely obliterated, the four blastomeres almost meeting in a point at the animal pole, while the ventral cross furrow is correspondingly longer. In Umbrella (Heymons '93) the dorsal and ventral furrows of this stage are parallel, i. e. are formed between the same cells *B* and *D*, the cells *A* and *C* being considerably separated. This is undoubtedly due to the presence of a large amount of yolk in the four blastomeres. Likewise in Planorbis (Rabl '79) and Neritina (Blochmann '81) we find the dorsal and ventral furrows of this stage similar to those of Umbrella, rather than Limax. These cross furrows are an invaluable aid in the determination of the axes of the later stages; the question of their relations and constancy will be discussed later.

FOURTH GENERATION. THIRD CLEAVAGE. EIGHT CELLS.

Plate I. Figs. 8-13; Plate II. Figs. 17-19; Plate III. Figs. 20, 21.

About two hours and a half intervene between the beginning of the four cell stage and that of the eight-cell stage. The third cleavage is accomplished by the division of the quartet of the third generation, *A*, *B*, *C*, *D*, into two superposed quartets (cf. Figure B, p. 52), $A^{4.1}-D^{4.1}$, and $a^{4.2}-d^{4.2}$. The series of stages shown in Figures 8–13 (Plate I.) represents the egg during this process. Figures 17 and 18 (Plate II.) give apical and lateral views respectively of an egg with the spindles of this generation. It will be noticed (Fig. 17) that the spindles in no case stand vertically, but that they are inclined toward the *right* (right and left being used as resident in the egg; see my earlier paper, '94, p. 180). The division of the chromatin elements in the spindles has just begun, and there is no trace of a constriction of the cytoplasm. A comparison of Figures 17 and 19 shows that the degree of obliquity of the spindles has increased during the interval between the two stages. Figure 19 represents a stage in which the constriction of the upper from the lower quartet, i. e. of the micromeres from the macromeres, has just been completed. The obliquity increases during the division, so that at its close the cells of the upper quartet lie in the furrows between the cells of the lower quartet. Thus it will be seen that this apparent shifting of the upper quartet upon the lower, known as the "spiral," takes place in large measure during the division of the cells. It will also be noted that the plane of division is at right angles to the axis of the spindle.

If we view the egg from the animal pole and pass from the lower derivative of a pair to the upper, we move in the direction of the hands of a clock, and therefore the division of this, the fourth generation, takes place in a *right* spiral, whereas that of the third presented the character of a *left* spiral. Applying the system of nomenclature which I have adopted to the derivatives of the third generation, we find that cells of the lower quartet will be designated by the exponent 4.1, and the upper by 4.2. It will be convenient in the further discussion of quartets to refer to them simply by their exponents, without reference to the individual cells of which they are composed. It will be seen from Figures 17 and 19 (Plate II.) that the dorsal and ventral cross furrows at the close of this stage do not lie at right angles to each other, as they did at the end of the four-cell stage, but that they cross each other at an angle as much less than 90° as is represented by the shifting of the cells to produce the spiral, i. e. they now cross at about 45°, as seen in the accompanying diagrams (Figures A and B).

FIGURE A.

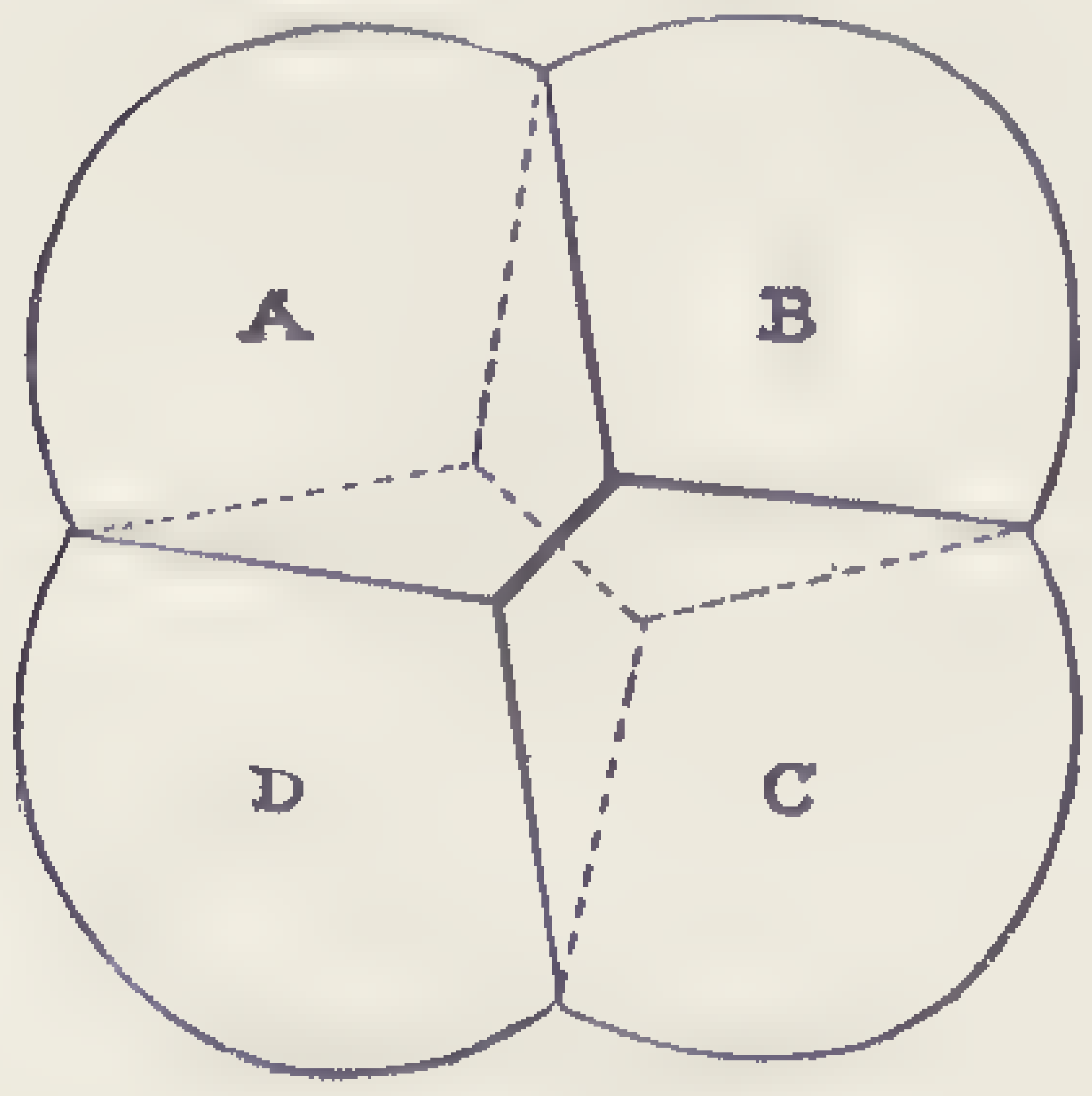

FIGURE B.

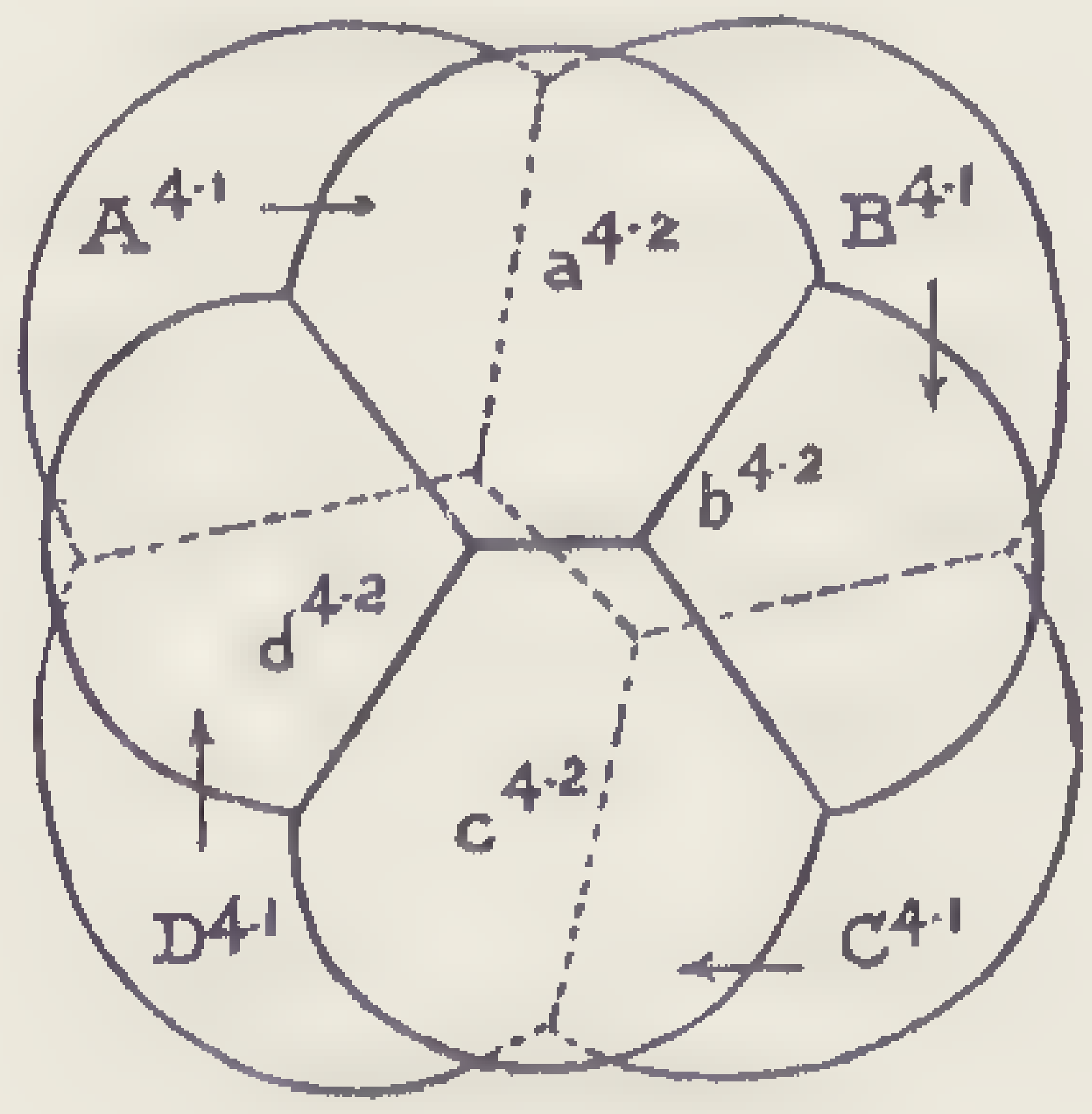

Figure A is a diagrammatic representation of the four-cell stage of Limax as seen from the animal pole, showing dorsal and ventral cross furrows. Figure B is the same of the eight-cell stage.

This condition is not quite realized in Figure 21 (Plate III.), owing to the near proximity of the succeeding division, which restores the cross furrows approximately to the conditions of the four-cell stage. Thus, in the typical eight-cell stage of Limax the cross furrows correspond to those of the same stage of Nereis (Wilson '92, Plate XIV. Fig. 11). In Umbrella likewise (Heymons '93, Taf. XIV. Fig. 4) the dorsal and ventral furrows are oblique to each other, crossing at about 45°, but differing in this important respect from the furrows of Limax and Nereis, that they are in this case formed by the juxtaposition of the cells of quadrants *B* and *D* at *both poles*, whereas in Limax and Nereis the ventral furrow only is formed by cells of these quadrants, the dorsal furrow being formed by $a^{4.2}$ and $c^{4.2}$, as is shown in Figure B. The furrows in Neritina (Blochmann ’81) are similar to those of Umbrella. The conditions in the eight-cell stage of Planorbis (Rabl ’79) are complicated by the fact that this pulmonate probably has reversed cleavage (cf. Rabl ’79, Taf. XXXII. Figs. 7, 9), and that therefore the mesoderm arises by a right spiral instead of a left one, as in the case of the unreversed or normal type of cleavage. Orienting the Planorbis egg for the purpose of comparison after the method employed by Wilson (’92) for Nereis, we have the first mesoderm cell arising from quadrant *C* instead of *D*; and in the four-cell and later stages the ventral cross furrow lies between *A* and *C* instead of between *B* and *D* (cf. Rabl ’79, Taf. XXXII. Figs. 7, 8 B, 11 B). The differences between Limax and Planorbis will be best shown by a comparison of Figures A and B with the corresponding stages of Planorbis given in the diagrams below.

FIGURE C.

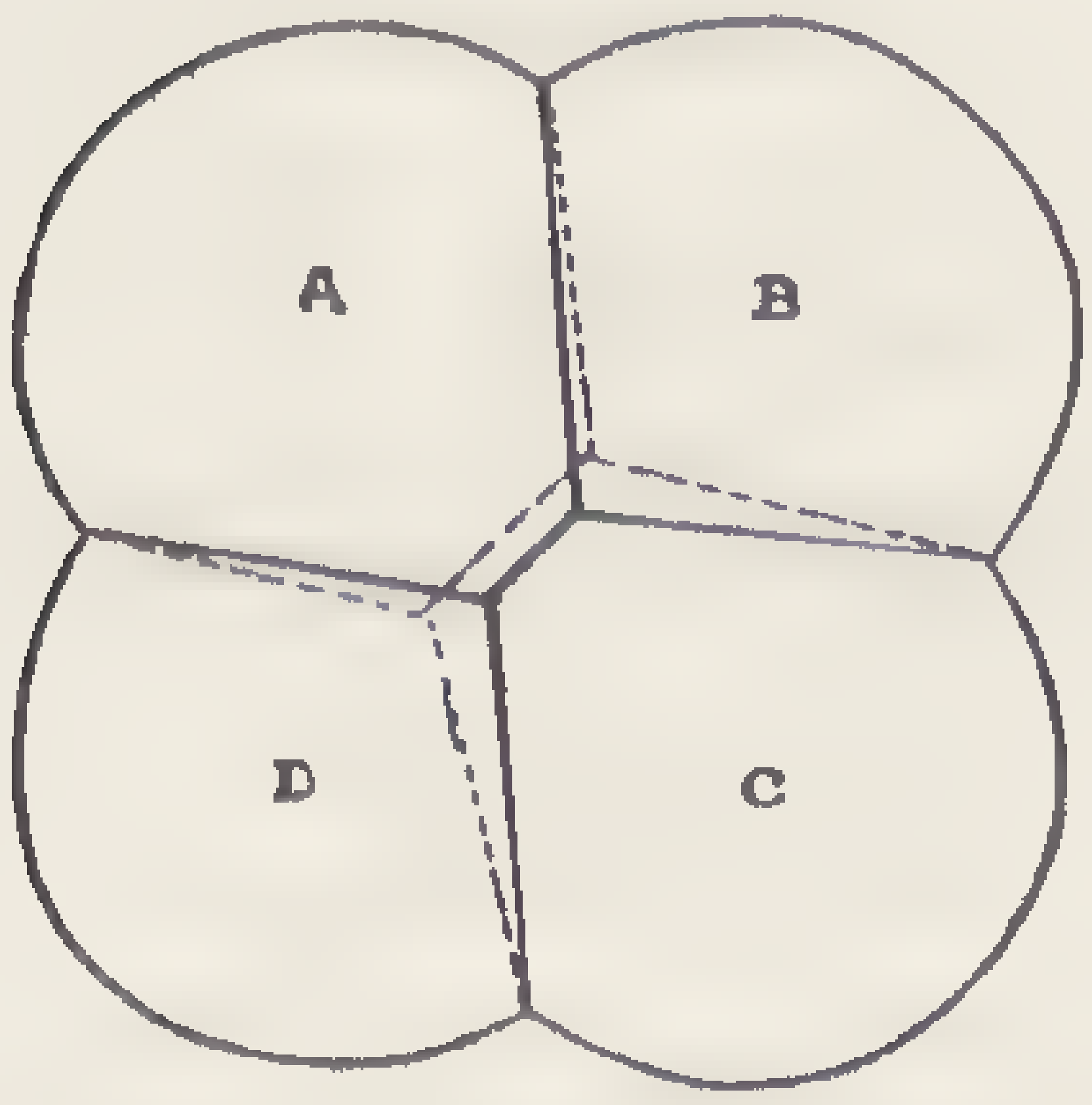

FIGURE D.

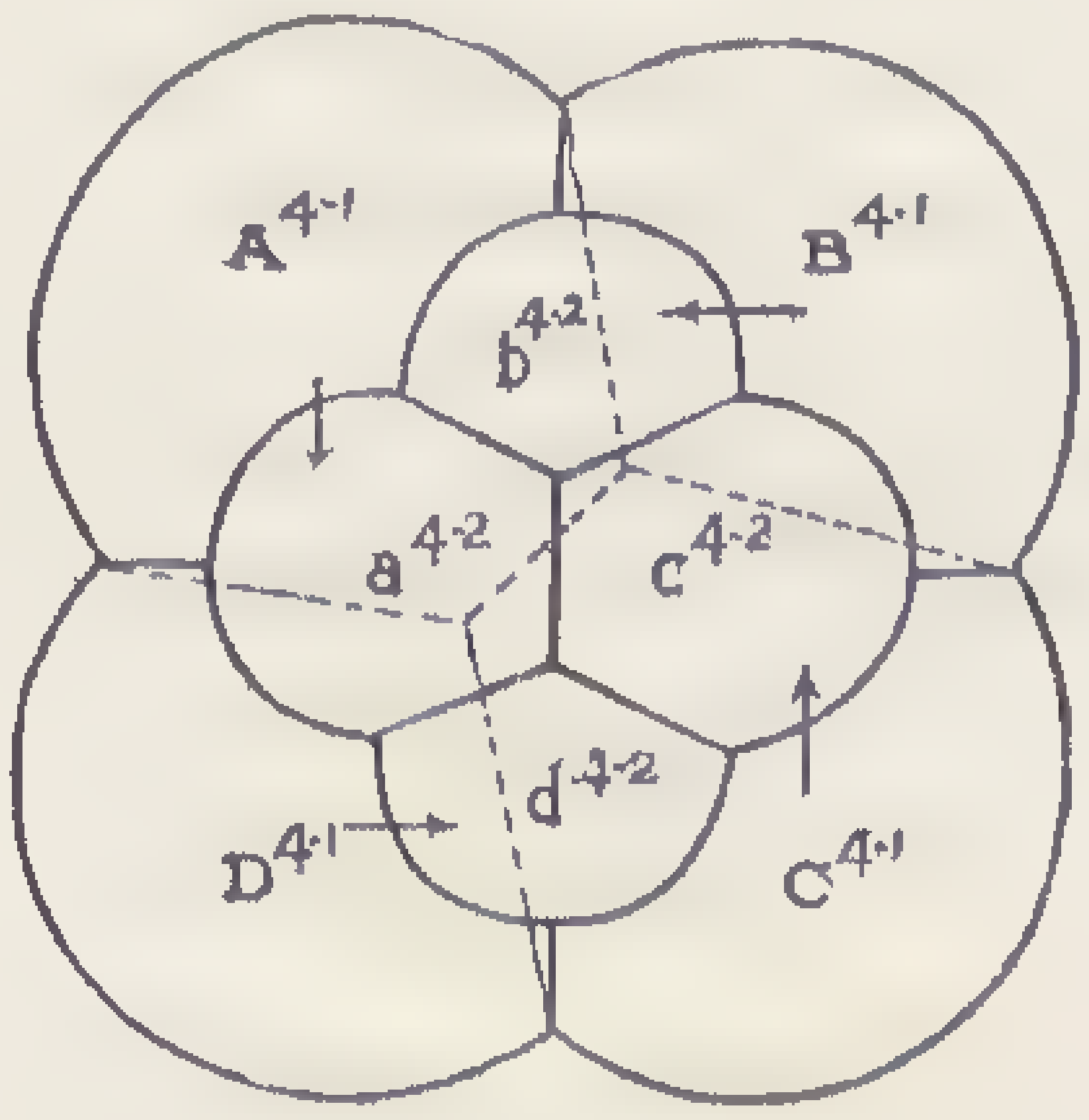

Figure C is a diagrammatic representation of the four-cell stage of Planorbis, showing dorsal and ventral cross furrows parallel. Combined from Rabl’s (’79) Taf. XXXII. Figs. 7–12. Figure D, the same of the eight-cell stage.

It will be seen that in Planorbis, as in Umbrella and Neritina, — all of them forms with considerable yolk, — the dorsal and ventral furrows are both formed by the contact of cells of the *same quadrants*. On the other hand, in Nereis and Limax the furrows of the two poles are formed by the contact of cells of *different* quadrants. In Nereis, however, the dorsal furrow is comparatively shorter than in Limax, and in Umbrella it is of still less extent.

I have observed no difference in the time of cleavage of the different quadrants. The nuclear conditions in Figure 19 (Plate II.) indicate that the division is very slightly more advanced in the posterior half of the egg, but there seems to be no acceleration of the division in the mesoderm-producing quadrant *D* over the quadrant *C* in this egg.

The division, as has been before stated, is in individual planes oblique to each other, and not in a common equatorial plane such as occurs in radial division. In Limax the cells of the two quartets 4.1 and 4.2 are of unequal size, the inequality being almost as great as in Nereis; this will be seen on a comparison of my Figure 19 with Wilson's ('92) Plate XIII. Fig. 10. We may therefore distinguish the components of the larger quartet as macromeres, and the smaller as micromeres of the first set. This difference in size, so marked at this period, persists in Nereis to a very late stage of development, but in Limax it is practically obliterated at the division leading to the next generation. Hence it is that a system of nomenclature based on these distinctions loses its significance when applied to the approximately equal cleavage of Limax.

FIFTH GENERATION. SIXTEEN CELLS.

With the formation of the two quartets of the fourth generation it has become no longer possible to designate a single cleavage furrow as producing the next generation. I shall therefore discuss the cleavage, from this time on, from the standpoint of the successive cleavage of quartets.

Division of Quartet 4.1, *forming* 5.1 *and* 5.2.

Plate III. Figs. 20, 21.

The basal and larger quartet of the eight-cell stage is seen in a mitotic condition in Plate III. Figs. 20 and 21. Here, as in the previous division, the spindles are not vertical, but much inclined; this time, however, the upper asters of each spindle lie to the *left*,[1] and not to the right, of the lower ones (Fig. 20). The division of the chromatin elements has already taken place in the spindles of this quartet (best seen in Fig. 21), and the conditions of the completed mitosis can readily be inferred from the figures. If we view the egg from the animal pole, and pass from the lower derivative to the upper, we move in a direction opposite to that of the hands of a clock, i. e. this division takes place in a left spiral. The division of this quartet (4.1) is almost equal (Plate III. Fig. 22), the basal derivatives (5.1) being but slightly larger than the upper ones (5.2). In this respect Limax differs from all the yolk-laden forms, — Neritina, Planorbis, Umbrella, and Nereis, — where the cells of the basal quartet (5.1) retain their preponderance, and may still be designated as macromeres after this division.

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[1] Cf. Kofoid '94, p. 180, for explanation of the use of right and left.

The cell $d^{5.2}$ (somatoblast of Wilson) is not appreciably larger than the other members of the quartet to which it belongs.

Division of Quartet 4.2, *forming* 5.3 *and* 5.4.

Plate III. Figs. 20–23; Plate IV. Figs. 28, 29.

The same stages which show spindles in the lower quartet also exhibit them in the upper and smaller quartet. The mitosis is not however so far advanced as in the lower quartet. The nuclear membrane can still (Figs. 20, 21) be traced, though the asters are present, and the axis of the spindle can therefore be determined. This, as in the lower quartet, is inclined; however, it is more nearly parallel to the equator than to the vertical axis. The inclination is in the *same general direction* as that of the spindles of the lower quartet, and the conditions of the completed division can be inferred as readily from the figures. Viewing the egg from the animal pole and passing from the lower derivative or aster to the upper, we move in a direction opposite to that of the hands of a clock, and this spiral, like that of the other quartet of this generation, is therefore a *left* one. This division, like that of the basal quartet, results in cells of almost equal size (Plate IV. Figs. 28, 29), the upper derivatives in this case (5.4) being, however, slightly larger than the lower (5.3).

The conditions in Figures 20, 21, show that the sixteen-cell stage will in this case follow immediately upon the eight-cell, without the intervention of a well marked twelve-cell stage. There is, however, so much variation in the rate of cleavage in Limax, that it might be expected that a twelve-cell stage would occasionally make its appearance. We have but to increase the difference between the mitotic conditions of the cells of the two quartets of Figures 20 and 21 to produce such a stage. Warneck ('50) figures in Tafel V. Fig. 46, a twelve-cell stage of Limax agrestis, and one egg in this stage has come under my own observation. This stage occurs regularly in forms with abundant yolk, as Planorbis, Umbrella, etc., but Nereis, like Limax, passes directly from the eight- to the sixteen-cell stage. The completed sixteen-cell stage is shown in Figure 22, Plate III., in which the genetic relations of the cells are still indicated by the approximated nuclei.

With the completion of the sixteen-cell stage and the fifth generation, the dorsal and ventral cross furrows are restored to the conditions of the four-cell stage, i. e. they cross each other at approximately right angles. A similar restoration to the conditions of the four-cell stage occurs in Nereis, also in Umbrella at the twelve-cell stage, and probably in Neritina.

In Planorbis, however, according to Rabl's ('79) interpretation, the cross furrow of the animal pole is not restored to the position of the four-cell stage, but is turned 90° from it (see his Taf. XXXII. Figs. 10 A, 11 A). To accomplish this it is necessary for each of the cells of the apical quartet (5.4) to be shifted 90° to the left, and thus completely out of their own quadrants over upon the adjoining quadrants. It seems very probable that Rabl is in error in this matter, and that in Planorbis, as in the other forms, the division of this generation results in the restoration of the cross furrows to the conditions of the four-cell stage.

An examination of the sixteen-cell stage (*cf.* Figs. 21, 22) shows that the cells of the apical quartet (5.4) lie in the same meridian as those of the same quadrant in the basal quartet, i. e. $a^{5.4}$ lies directly over $a^{5.1}$, $b^{5.4}$ over $b^{5.1}$, etc.; $a^{5.2}$, $b^{5.2}$, etc. lie to the left of the meridian of the quadrant to which they belong, and $a^{5.3}$, $b^{5.3}$, etc. to the right. Thus of the four granddaughter cells of the original blastomere occupying the quadrant, two only occupy the meridian corresponding to the middle of the quadrant, the other two being placed laterally to it, one upon either side. Similar conditions obtain in the corresponding stage of Nereis. In Neritina, Planorbis, and Umbrella, the fact that the twelve-cell stage is succeeded by the twenty-four-cell stage obscures somewhat the typical arrangement, though it can still be traced. It will readily be seen that, when the disturbing elements of unequal and non-synchronous division are removed, this arrangement of the four granddaughter cells will hold good for the descendants of *any* blastomere in spiral cleavage, and that normal and reversed cleavage will differ only in the transposition of the lateral granddaughter cells; e. g. in the case under discussion $a^{5.2}$ and $a^{5.3}$ would be in a case of reversed cleavage transposed. Owing, perhaps, to the unequal distribution of the yolk, this typical arrangement is not found in the sixteen-cell stage of Chiton as figured by Kowalevsky ('83) and Metcalf ('93), though it can be traced in the later stages. Metcalf says of this phenomenon, "*Each* cell then lies in the same meridian as its grandparent, — a fact shown more clearly in the cleavage of such eggs as those of Nereis and Crepidula." It is at once evident that this is but a partial and misleading statement of the case, since it ignores the fact that there are *four granddaughter* cells of every blastomere. It has its explanation in the confusing custom adopted in previous systems of nomenclature of regarding the larger of two daughter cells as the mother, the smaller as the daughter. In reality both are daughter cells, and the mother cell has passed out of existence with their origin, a fact which a logical system of nomenclature of *cell lineage* must always recognize.

SIXTH GENERATION. THIRTY-TWO CELLS.

Following the formation of the sixteen cells of the previous generation there comes the usual rounded condition in which each blastomere asserts its individuality and diverges from its nearest of kin. This in turn is followed by the flattened condition, in which the spherical contour of the egg as a whole is restored. It is during this period that the spindles which begin the formation of the sixth generation first appear. As in the previous generation there was a lack of synchronism in the cleavage of the two quartets 4.1 and 4.2, as shown in the nuclear conditions of Figures 20 and 21 (Plate III.), so here there is a similar separation of the divisions of this generation into two mitotic periods, the first appearing in the largest cells of the embryo, viz. the two quartets at the vegetative pole, and resulting in the twenty-four-cell stage; the second involving the animal hemisphere, and resulting in the thirty-two-cell stage, thus realizing in this stage Warneck's ('50) conclusion that cleavage progresses according to the age of the cleavage spheres. The first phase is separated from the second by a period in which all the nuclei are in a resting condition. This period lasts some hours, and hence it is that cleavage stages killed at random contain a large proportion of eggs in the twenty-four-cell stage.

Division of Quartet 5.1, *forming* 6.1 *and* 6.2.

Plate III. Figs. 23-25; Plate IV. Figs. 27-32; Plate V. Figs. 33-36.

Figure 23 (Plate III.) shows a lateral view of an egg at the completion of the first mitotic phase. The quartet 5.1 has divided, forming 6.1 and 6.2. Applying the test as in previous generations, it is readily seen that this is a right spiral. The remnants of the nuclear spindle and the asters leave no question as to the relationship of the cells in this egg. The upper derivative lies to the right of the lower one in every case. Reference to the quartets 6.1 and 6.2 in Figures 27–29, 31–34, 36, of four other eggs, shows the constancy of the direction of this spiral. The division in this quartet is about equal, and is synchronous in the four quadrants.

Division of Quartet 5.2, *forming* 6.3 *and* 6.4.

Same Figures as for 5.1.

Figure 23 shows the quartet at the close of the division which has resulted in the formation of the quartets 6.3 and 6.4. The nuclear conditions in like manner show that this division has also taken place in a right spiral. The remnants of the spindles are parallel to those of the quartet 5.1, and the upper derivative lies to the right of the lower one. These divisions are also approximately equal and synchronous. The arrangement of the cells of the vegetative pole is very regular. The ventral cross furrow remains approximately at right angles to the dorsal furrow. The two cells $b^{6.1}$ and $d^{6.1}$ meet in this ventral furrow, and are hexagonal in outline, while the other two members of the quartet $a^{6.1}$ and $c^{6.1}$ are pentagonal. All four cells of the quartet 6.2 are hexagonal, as will be seen in Plate IV. Figs. 27–29, and 31. A comparison of the quartet 6.1 of Figure 27 with 5.4 of Figure 28 shows how little difference there is in the size of the cells of the two poles in the superficial view. In optical section, however, a distinct difference can be detected in the size of the deeper lying parts of these cells. In Figure 30 (Plate IV.) is represented such a section taken from the egg in the position shown in Figure 29. The section passes through the vertical axis in the plane corresponding to that of the paper in Figure 29, thus cutting the ventral cross furrow at right angles, and passing through the quadrants *b* and *d*. It will be seen that one of the two cells of the vegetative pole, labelled $d^{6.1}$ in the drawing, is much larger than the other, $b^{6.1}$. This is the cell which at the next generation gives rise to the first mesoderm cell, $d^{7.2}$, or *M*.

The generalization which Rabl made in his paper on Planorbis ('79), — "dass bei den Keimen mit reichlichem Nahrungsdotter von dem Zeitpunkte an, als Aequatorialfurchen auftreten, die Zellvermehrung in arithmetischer, bei den Keimen mit spärlichem Nahrungsdotter dagegen zuerst in arithmetischer, sodann aber in geometrischer Progression erfolgt," — is not borne out by the cleavage in Limax, as the twelve-cell stage does not regularly occur. The cleavage in Limax runs,

4 . . 8 . . . 16 . . . . 24 . . . . 40.
or, (4) : (4) : (4) + (4) : (4) + (4) : (4) + (4) + (4) + (4).

This is fundamentally an arithmetical progression, a series whose common difference is four, the mathematical expression of the increase in the number of cells in the spiral type of cleavage. It varies, however, from the strict arithmetical series in that a multiple of the common difference is sometimes added as a result of the approximated or even synchronous division of two or more quartets. Thus the series may in some of its parts, like the twelve- to twenty-four-cell period of Planorbis, take the form of a geometrical series; but this is not fundamental, and, as Warneck in 1850 expressed the law, "In jedem Stadium des Furchungsprocesses entstehen nur vier Furchungskugeln, d. h. die Theilung geht nicht in einer geometrischen sondern einer arithmetischen Progression vor sich."

The general contour of the egg in the twenty-four-cell stages figured (Plate III. Figs. 23–25, Plate IV. Figs. 27–32, Plate IV. Figs. 33, 34) has been spherical, the transverse and vertical diameters being approximately the same. In the period leading up to this stage, however, eggs are found showing a considerable flattening in the dorso-ventral direction, i. e. a shortening of the vertical axis. In one egg showing the flattening, the dorso-ventral diameter was 70 μ and the transverse 120 μ. This change may be dependent upon an elimination of such a cleavage cavity as is shown in Plate V. Fig. 34. A similar flattening is often found in those eggs in which the divisions leading to the forty-cell stage are taking place. These divisions will now be discussed. The order of their discussion does not, however, indicate their chronological succession.

Division of Quartet 5.3, *forming* 6.5 *and* 6.6.

Plate VI. Figs. 37, 39, 41.

The division of this quartet does not take place, in some instances at least, until after the forty-cell stage; i. e. it is accompanied by divisions of the succeeding generation in other quartets. See Figures 39 and 41. In Figure 39 the cells $a^{5.3}$ and $d^{5.3}$ have divided, and $b^{5.3}$ is in a mitotic state, but $c^{5.3}$ is as yet undivided. In $b^{5.3}$ the end plates have been formed, but the cytoplasm is not yet constricted. The axis of the spindle lies parallel to the plane of the equator. There is every indication that the division is very nearly meridional. Figure 39 has forty-two cells; in Figure 41 there are forty-five cells; here, however, it is cells $a^{5.3}$ and $d^{5.3}$ that have but recently divided, the other cells of the quartet having evidently been divided for some time. Thus there is no constancy as to the sequence in which the cells of this quartet divide. In Figure 41 (Plate VI.) this division still shows some slight traces of a right spiral. Figure 37 (Plate VI.), a forty-cell stage, is described in my notes as containing the cells $a^{6.6}-d^{6.6}$, $a^{6.5}-d^{6.5}$, but owing to the rotundity and opacity of this egg the details in the periphery, i. e. the region of the cells in question, are very much obscured, and I consider this determination questionable.

The comparison of the division of this quartet with the corresponding one in Nereis is very interesting. In Nereis, as in Limax, the division is *nearly meridional*, and with traces of a right spiral. In this instance it takes place at the twenty-nine-cell stage, and the products form the *prototroch*. In Umbrella it does not take place till the sixty-five-cell stage, and here also exhibits an obscure right spiral. (See Tables of Cleavage, pp. 66, 74, and 75.)

Division of Quartet 5.4, *forming* 6.7 *and* 6.8.

Plate V. Figs. 35, 36; Plate VI. Figs. 37, 39, 41.

This is one of the first divisions to follow the twenty-four-cell stage. Figures 35 and 36 (Plate V.) show it in progress; Figures 37, 39, and 41 after completion. It takes place in a very evident right spiral, the upper aster and derivative lying to the right in every case. The division is approximately equal, but is not synchronous in the different quadrants, as is shown in Figures 35 and 36. In Figure 35 all the cells of this quartet have divided except $a^{5.4}$; the quadrant *c*, judging from the size and position of the daughter nuclei, has evidently led in the division. In Figure 36, $b^{5.4}$ is the only one which has divided, resulting in $b^{6.7}$ and $b^{6.8}$, the other cells containing spindles. It is evident from these two cases that it is impossible to predicate any regular sequence in the successive divisions of the quadrants of this quartet.

This completes the discussion of the cleavages of this generation. It will be noted that all of the divisions clearly take place in a right spiral, with the exception of that of 5.3, and that this, though predominantly meridional, still shows traces of a right spiral.

SEVENTH GENERATION. SIXTY-FOUR CELLS.

As was stated in my earlier paper ('94, p. 188), the divisions of this generation begin before those of the sixth are completed.

Division of Quartet 6.1, *forming* 7.1 *and* 7.2.

Plate VI. Figs. 38, 40, 42.

The division of this quartet is the point of greatest interest in the cleavage, as it results in a differentiation of the germ layers, or at least in a separation of the primary mesoderm from the ect-entoderm.

In spite of the examination of a large number of eggs, and the repeated killing of those whose age and approximate stage were known, I have not been able as yet to obtain an egg showing the *spindles* resulting in this division. Figures 38, 40, and 42 (Plate VI.) all represent stages subsequent to the formation of M, and the other members of the quartet 7.2. There is some evidence, however, as to the character of the division. Figure 38 is a view of the vegetative pole of an egg of forty cells. The four central cells have *seven* peripheral neighbors. Deeper focusing reveals the presence of a large nucleus, lying within a definite cell boundary. This is quite below the level of the nuclei of the vegetative quartet. Its nucleus lies below and slightly nearer the median plane than that of $d^{7.1}$. The superficial extent of this deeper lying cell is limited to a narrow strip adjoining the cell with which it has arisen, i. e. it is peripheral to $d^{7.1}$. The other members of the quartet 7.2 are present, and when the test for the spiral is applied it is evident that this is a *left spiral, though the amount of the shifting* is evidently not very great. It is quite plain that in this case the cell $d^{7.2}$, which gives rise to the mesoderm, comes from $d^{6.1}$ at the time of its division into $d^{7.1}$ and $d^{7.2}$. As in previous stages, the quartets d and b are in contact at the ventral cross furrow.

Figure 40 (Plate VI.) represents a forty-two-cell stage which has recently been the scene of a number of divisions. Here, as in the egg just discussed, the cell $d^{7.2}$ is very large, and is crowded in toward the centre of the egg, lying below $d^{7.1}$ and slightly nearer the median plane. It maintains a small crescentic connection with the exterior, between $d^{7.1}$ and $d^{6.3}$. The other members of the quartet 7.2 are present, and show about the same nuclear conditions that $d^{7.2}$ ($= M$) does. They are therefore of about the same age. The divisions of the cells of the quartet 6.1 in this egg have evidently been very nearly equatorial, and unless there should be at a subsequent period some shifting of the mesoderm to a position nearer to the median plane, — as adopted in this paper, — it would be necessary to orient this egg as Rabl and Blochmann have oriented Planorbis and Neritina. Judging from the nuclear conditions the division has taken place quite recently. Eggs of later stages show that the mesoderm is generally placed bilaterally with reference to the cross furrows and the prevailing quadrangular form of the egg of those stages. The division of this quartet is in all cases unequal, the smaller cells lying at the vegetative pole.

The conditions of the egg shown in Figure 42 (Plate VI.) were for a long time very puzzling to me. It contains forty-five cells, which readily group themselves into quartets. A median deep-lying mesoderm cell is present, as in the egg last described, but the relations of the cells of the quartet of the vegetative pole to this cell and to one another are different from those of the other eggs, in the following respect: the cells which meet in the ventral cross furrow are $a^{7.1}$ and $c^{7.1}$, instead of $d^{7.1}$ and $b^{7.1}$, as in the other two eggs figured. Repeated trials failed to give any other solution which would accord with the conditions in the other parts of the egg. The juxtaposition of the quadrants *a* and *c* occurs normally in the reversed type of cleavage (see Figs. C and D, p. 53), and the possibility is at once suggested that this egg may have had reversed rather than unreversed or normal cleavage. Other parts of the egg, however, furnish no corroborative evidence, and the suggestion must be dismissed. I believe, then, that owing to some cause, mechanical or other, a change in the normal relations of the cells of the quartet to one another has been brought about. This has naturally raised the question as to the constancy of the cross furrows, upon which the orientation of the egg so largely depends. This case in Limax is not an isolated one, for in Nereis, where, as has already been pointed out, the dorsal furrow is formed in the early stages of cleavage by the apposition of the quadrants *a* and *c*, we find this normal arrangement disturbed in one instance, the furrows being formed by the quadrants *b* and *d* (Wilson '92, Plate XIV. Fig. 19, p. 390). In the later stages, i. e. after the cells of the seventh generation appear at the animal pole, the dorsal cross furrow is normally formed by the apposition of *b* and *d*, but in one case (Wilson *l. c.*, Plate XVI. Fig. 35) we find this arrangement disturbed, the cross furrow being formed by *a* and *c*. This disturbance is also accompanied by the mitotic conditions of neighboring cells.

Another case occurs in Neritina (Blochmann '81, Taf. VII. Figs. 51, 53, 56), in which two eggs — one a thirty-six-, the other a forty-cell stage — present cross furrows formed by the apposition of different pairs of quadrants. There is not here, as in Nereis, an intervening mitosis to explain the disturbance of the customary order.

In Lang's ('85) Taf. 34, Figs. 14, 15, we find a similar transposition from the usual arrangement of the apical quartet, accompanied in this case by mitosis in adjoining cells. In view of these cases it seems not improbable that there has been in this Limax egg a disarrangement of the normal condition at the vegetative pole, as a result perhaps of the recent divisions at that pole, the collapse of the cleavage cavity, or some other mechanical disturbance.

It seems almost certain that the primary mesoderm cell, $d^{7.2}$ (M), is formed synchronously with the other members of the quartet to which it belongs. In this respect Limax stands in sharp contrast to Nereis, where the primary mesoderm cell originates at the thirty-eight-cell stage, but the cleavage of the remaining cells of the quartet is long delayed. Also in Umbrella there is a corresponding lack of synchronism, for the division of this quartet commences with the formation of $d^{7.2}$ at the twenty-five-cell stage, but is not completed until the forty-seven-cell stage is reached. Likewise in Planorbis the formation of the primary mesoblast antedates the cleavage of the other cells of the same quartet.

Division of Quartet 6.2, *forming* 7.3 *and* 7.4.

Plate IV. Figs. 31, 32; Plate V. Fig. 35; Plate VI. Figs. 38, 40.

The spindles resulting in this division are among the first to appear in the twenty-four-cell stage. Figures 31 and 32 (Plate IV.) show spindles in all of the cells of this quartet except $a^{6.2}$, and in this the stages preparatory to the formation of the spindle are seen (Fig. 31). The nucleus is very large; the chromatic granules are distributed in a network, and the nuclear membrane is still intact. At diametrically opposite sides of the nucleus, in the long axis of the cell, and closely applied to the nuclear membrane, there are two large, clear spherical spaces in the cytoplasm, bounded by a granular zone. These structures are probably the astrocœls of Fol ('91). The surface of the nucleus presents on one side a peculiar constriction, or crease, running between the two astrocœls, as though they were connected by a strand of substance (central spindle) which was compressing the thin nuclear membrane.

The spindles in this quartet, as shown in Figures 31 and 32 (Plate IV.) are almost free from any inclination indicative of a spiral arrangement. The one in $b^{6.2}$ shows traces of a right spiral, but there is indication from the position of the spindles that the division will be equatorial rather than oblique; such indeed is the character of the division, as is shown in $c^{7.3}$, $c^{7.4}$, Figure 35 (Plate V.). The order of nuclear advancement in this quartet as shown in Figure 32 (Plate IV.), is *b*, *d*, *c*, *a*, but in Figure 35 (another egg) the cell *c* has been the first to divide, whereas in Figure 38 (Plate VI.), a forty-cell stage, the cell $d^{6.2}$ is just dividing, it being the last of its quartet to undergo the process. This seems to show either that the cleavage in this quartet progresses very slowly, or, what is more probable, that there is considerable variation in the sequence in which its components divide. In the case of Figure 38 (Plate VI.) there is strong indication of a *left* spiral; so also a slight indication of the same in Figure 40, $a^{7.3}$, $a^{7.4}$. In all the other figures the division seems to be *equatorial.* A comparison with Nereis reveals in this instance the same marked agreement noted for the meridional cleavage of the quartet 5.3. In Umbrella this division takes place at the twenty-nine-cell stage, and is also equatorial.

Division of Quartet 6.3, *forming* 7.5 *and* 7.6.

Plate VI. Figs. 41, 42.

This division is in progress in the quadrant *c* in Figures 41, 42, and the other members of the quartet are also approaching mitosis. There is a faint trace of a *left* spiral to be detected in the position of $c^{7.5}$ and $c^{7.6}$ of Figure 41, but the division is predominantly equatorial.

Division of Quartet 6.4, *forming* 7.7 *and* 7.8.

Plate VI. Figs. 39, 40, 41, 42.

In the two eggs figured the spiral is clearly shown by the relative position of the nuclei to be a *left* spiral. Thus all the spirals of this seventh generation, wherever they have been traced, have been *left* spirals.

With this forty-five-cell stage my detailed account of the cleavage closes. I have not been able to decipher satisfactorily the conditions in the eggs of the next stage, because during this stage a large number of cells divide, — in one instance as many as thirteen. Moreover, the rounded contours of the mitotic cells produce such changes in the surface of the egg as effectually to obscure all trace of its poles, and the absence of polar globules, of macromeres, or of any "landmarks" whatever for orientation, makes any interpretation of these later stages at the best provisional, and very largely conjectural. Added to these difficulties is that produced by the vacuolation which prevailed in a very large proportion of the eggs which I have examined. This distorts and obscures the relation of cells to such an extent as to make a determination of their lineage extremely difficult, if not impossible.

As late, however, as the hundred-cell stage, when four mesoderm cells are present, it is possible on favorable eggs to work out a provisional lineage, but I have not as yet succeeded in connecting this with the forty-five-cell stage.

Thus the outcome of my work as a study in *cell lineage* is a disappointment, for I have not been able to trace a single blastomere to a definite organ of the adult. At the stage of thirty-eight cells in Nereis, Wilson was able to assign a definite fate to each blastomere; but in Limax there is no trace, save in the early differentiation of the mesoderm, of that precocious development so marked in Nereis. This fact makes the identity of the cleavage of Limax with that of Nereis all the more wonderful and difficult to explain.

I insert here (p. 66) a table of the cleavage of Limax, so far as I have followed it, which epitomizes the foregoing discussion of the alternation of spirals in successive generations of cells. The spirals, wherever they occur, conform to the law of alternation as defined in my former paper ('94, p. 189).

C. Literature on Spiral Cleavage.

The conformity of other animals to the law of spiral cleavage has in all cases been obscured by the systems of nomenclature employed. Since no one of my predecessors has formulated this supposed law, it of course has not been tested on any of the forms whose cleavage has been worked out. It has seemed desirable, therefore, to go over the available literature and point out those cases which agree, and those which seem to disagree with my proposition.

In order that the subject may be treated in as brief a form as possible the discussion of each case is accompanied by a tabulated presentation of the cleavage, in which the author's designation of cells and spirals is joined in parallel columns with the designation which my system would impose.

In my former paper ('94, pp. 192–196) the conformity of the cleavage of Neritina, as described by Blochmann ('81), to the alternation of spirals was discussed, and the cleavage tabulated. In what follows I have discussed all other cases which seemed worthy of consideration in this connection.

Fol states ('75, p. 117) that Clio likewise has the same regular cleavage as Cavolina, and his few figures of the early stages of this form suggest that the cleavage is of the normal type. Cymbulia also seems to conform to this type.

The cleavage of the Heteropods, which he ('76) states is identical with that of the Pteropods, is, according to his figures, of two types: Firoloides (Plate I. Figs. 1–3) presenting the normal type, Pterotrachea (Plate IV. Figs. 5, 6) the reversed type, *if his labelling, indicating the lineage, is correct.* There is evidence, however, that some of the divisions belong to the normal type (Plate IV. Fig. 9).

CLEAVAGE OF LIMAX.

Generation.	Number of Cells.	Designation of Cells.	Spirals.
VII.	40	$a^{6.4} < \left. \begin{matrix} a^{7.8} \\ a^{7.7} \end{matrix} \right\}$	Left.
	48	$a^{6.3} < \left. \begin{matrix} a^{7.6} \\ a^{7.5} \end{matrix} \right\}$	Left, — cleavage nearly equatorial.
	28	$a^{6.2} < \left. \begin{matrix} a^{7.4} \\ a^{7.3} \end{matrix} \right\}$	Left, — cleavage nearly equatorial.
	36	$a^{6.1} < \left. \begin{matrix} a^{7.2} \\ a^{7.1} \end{matrix} \right\}$	Left.
VI.	32	$a^{5.4} < \left. \begin{matrix} a^{6.8} \\ a^{6.7} \end{matrix} \right\}$	Right.
	44	$a^{5.3} < \left. \begin{matrix} a^{6.6} \\ a^{6.5} \end{matrix} \right\}$	Right, — cleavage nearly meridional.
	24	$a^{5.2} < \left. \begin{matrix} a^{6.4} \\ a^{6.3} \end{matrix} \right\}$	Right.
	20	$a^{5\ 1} < \left. \begin{matrix} a^{6.2} \\ a^{6.1} \end{matrix} \right\}$	Right.
V.	16	$a^{4.2} < \left. \begin{matrix} a^{5.4} \\ a^{5.3} \end{matrix} \right\}$	Left
	12	$A^{4.1} < \left. \begin{matrix} a^{5.2} \\ a^{5.1} \end{matrix} \right\}$	Left.
IV.	8	$A < \left. \begin{matrix} a^{4.2} \\ A^{4.1} \end{matrix} \right\}$	Right.
III.	4	$A, B, C, D.$	Left.
II.	2	$\overline{AB}, \overline{CD}.$	
I.	1	Ovum.	

As stated in my earlier paper ('94, p. 191), there are some indications in Rabl's work that Planorbis presents a case of reversed cleavage, i. e. the cleavage is such that the spirals of the even generations are left, and those of the odd right. Fol's ('80) figures of the eight- and sixteen-cell stages of this Pulmonate (Plate IX.–X. Figs. 1, 2) indicate that the spiral of the fourth generation is a left one, and that of the fifth a right one. My own sketches of Planorbis, made from living eggs in the spring of 1892, show the same direction in these spirals. So far as can be judged from these facts, the alternation of spirals holds in this case, which is probably one of reversed cleavage.

CLEAVAGE OF CAVOLINA.

Fol ('75).

Fol's Nomenclature.		Generation.	Number of Cells.	Revised Nomenclature.	
Spirals.[1]	Cells.			Cells.	Spirals.
Left.	$\begin{matrix} I' \\ I'' \end{matrix} > I'$	VI.	20	$a^{5.2} < \begin{matrix} a^{6.4} \\ a^{6.3} \end{matrix}$	Right.
Right.	$\begin{matrix} 1 \\ 1' \end{matrix} > 1$	V.	16	$a^{4.2} < \begin{matrix} a^{5.4} \\ a^{5.3} \end{matrix}$	Left.
Left.	$\begin{matrix} I' \\ I \end{matrix} > I$		12	$A^{4.1} < \begin{matrix} a^{5.2} \\ a^{5.1} \end{matrix}$	Left.
Right.	$\begin{matrix} 1 \\ I \end{matrix} > I$	IV.	8	$A < \begin{matrix} a^{4.2} \\ A^{4.1} \end{matrix}$	Right.
	I, II, III, IV.	III.	4	*A, B, C, D.*	Left. Division oblique, cells in one plane.

If the reversed spirals persist until the formation of the mesoderm, this germ layer would then come from the *right* posterior macromere, instead of the left posterior one, i. e. if we adopt Wilson's orientation instead of Rabl's. In the table given above it will be noticed that the mesoderm cell *M* is placed as a member of the basal quartet of the sixth generation. It should be remembered, however, that Rabl in a later paper ('80) revised this earlier opinion as to the origin of the mesoderm cell, deriving it this time by division from the cell *M* of the above table. According to this the mesoderm cell in Planorbis, as in many other forms, belongs to the quartet 7.2. It seems to me that the orientation of the stages of Planorbis previous to the formation of the mesoderm is still an open question. As I have stated in the discussion of Limax, the orientation

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[1] Fol does not name or discuss spirals. The nomenclature in this column is deduced entirely from his lettering of the cells. After the third generation the divisions of only one quadrant (*I*) are followed. In this, as in the following tables, capital letters indicate macromeres, and the lineage of only a single quadrant (*a* of the revised nomenclature) is given.

CLEAVAGE OF PLANORBIS.

Rabl ('79).

RABL'S NOMENCLATURE.	Generation.	Number of Cells.	REVISED NOMENCLATURE.	
Cells.			Cells.	Spirals.
$\left.\begin{matrix} E_5 & E_6 & E_7 & E_8 \\ E_{17} & E_{18} & E_{19} & E_{20} \end{matrix}\right\}$	VI.	24	$a^{5.2} < \begin{matrix} a^{6.4} \\ a^{6.3} \end{matrix}$	Left ?
$\left.\begin{matrix} E_{13} & E_{14} & E_{15} & E_{16} \\ EJ_1 & EJ_2 & EJ_3 & M \end{matrix}\right\}$		24	$A^{5.1} < \begin{matrix} a^{6.2} \\ A^{6.1} \end{matrix}$	Left.
$\left.\begin{matrix} E_1 & E_2 & E_3 & E_4 \\ E_{12} & E_9 & E_{10} & E_{11} \end{matrix}\right\}$	V.	24	$a^{4.2} < \begin{matrix} a^{5.4} \\ a^{5.3} \end{matrix}$	Right.
$\left.\begin{matrix} E_6 & E_7 & E_8 & E_5 \\ EJ_1 & EJ_2 & EJ_3 & ME \end{matrix}\right\}$		12	$A^{4.1} < \begin{matrix} a^{5.2} \\ A^{5.1} \end{matrix}$	Right.
$\begin{matrix} E_1 & E_2 & E_3 & E_4 \\ EJ_1 & EJ_2 & EJ_3 & ME \end{matrix}$	IV.	8	$A < \begin{matrix} a^{4.2} \\ A^{4.1} \end{matrix}$	Left.
$EJ_1\ EJ_2\ EJ_3\ ME$	III.	4	$A, B, C, D.$	Right.
	II.	2	$\overline{AB}, \overline{CD}.$	
	I.	1	Ovum.	

of the early stages depends on the estimated amount of shifting of the mesoderm cell upon the basal quartet $a^{7.1} - d^{7.1}$. There is evidence in Rabl's figures ('80, Taf. XXIX. Figg. 2, 3, 4) that there is at least some shifting, but whether it is sufficient to justify an orientation similar to that adopted by Wilson for Nereis cannot be decided from the evidence at hand. It is however sufficient to raise the question as to the correctness of Rabl's orientation.

In Planorbis the asymmetry of the adult is sinistral; so also in Physa heterostropha, whose segmentation likewise shows the same phenomenon of reversed cleavage, as I can affirm from my own observations on the living egg.[1] In my former paper ('94, p. 191) I called attention to the fact that Haddon ('82, Plate XXXI. Fig. 6) figures a four- to eight-cell stage of Janthina which apparently presents a left spiral, i. e. the reverse of that found in a corresponding stage of Limax and other dextral forms. But according to Fischer ('80–87, p. 775) Janthina is a *dextral* form. These cases of Planorbis, Physa, and Janthina at once raise a most interesting question as to the relation that may exist between the two forms of cleavage, normal and reversed, and the dextral or sinistral asymmetry of the adult. The occurrence of reversed cleavage in these two sinistral forms is suggestive of a causal relation between the conditions of the embryo and the adult, but the existence of this one observation of Haddon's is sufficient to throw much doubt on that conclusion. There remains the possibility that on renewed examination the cleavage of Janthina may prove to be normal, and that this case of Haddon's may be referred to a chance sinistral form. In answer to an inquiry of mine, Dr. J. I. Peck of Williams College has kindly written, "I have never seen a sinistral Janthina; all of those which came under my observation ('93) were dextral: all of those in our collection here are dextral also, as were those I saw at the Museum at Yale. These were of four species, I think, and collected at different points of the Atlantic and Pacific, both Arctic and Tropical. I have, however, probably not seen enough material to expect a sinistral individual." Of the two or three hundred shells of Janthina, representing several species, in the Museum collection, not one is sinistral.

The data for the discussion of this interesting question are as yet too scanty to justify any generalizations. The examination of the cleavage in such genera as Fulgur and Achatinella, which contain both dextral and sinistral forms, as well as that of heterostrophic species, ought to be decisive upon this point, where we have as yet so little light.

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[1] Consult also the Addendum, page 111.

CLEAVAGE OF APLYSIA.

Blochmann ('83).

Blochmann's Nomenclature.		Generation.	Number of Cells.	Revised Nomenclature.	
Spirals.	Cells.			Cells.	Spirals.
—	$> b_2'$	VII.	—	$a^{6.3} < \begin{matrix} a^{7.6} \\ a^{7.5} \end{matrix}$	Left.
—	$\begin{matrix} b_3' \\ b_3 \end{matrix} > b_3$		—	$a^{6.2} < \begin{matrix} a^{7.4} \\ a^{7.3} \end{matrix}$	Meridional, slightly left.
—	$> b$		—	$A^{6.1} < \begin{matrix} a^{7.2} \\ A^{7.1} \end{matrix}$	Left.
—	$\begin{matrix} b_2 \\ b_2' \end{matrix} > b_2$	VI.	20	$a^{5.2} < \begin{matrix} a^{6.4} \\ a^{6.3} \end{matrix}$	Right.
—	$\begin{matrix} b_3 \\ b \end{matrix} > b$		20	$A^{5.1} < \begin{matrix} a^{6.2} \\ A^{6.1} \end{matrix}$	Right.
Left.	$\begin{matrix} b_2 \\ b \end{matrix} > b$	V.	12	$A^{4.1} < \begin{matrix} a^{5.2} \\ A^{5.1} \end{matrix}$	Left.
Right.	$\begin{matrix} b_1 \\ b \end{matrix} > b$	IV.	8	$A < \begin{matrix} a^{4.2} \\ A^{4.1} \end{matrix}$	Right.
	b, *a*, *d*, *c*.	III.	4	*A*, *B*, *C*, *D*.	Left.
	A′, *A*.	II.	2	$\overline{AB}$, $\overline{CD}$.	

In the above table the labelling of cells indicated in the second column is that given by Blochmann to cells which seem to be homologous with those of quadrant *A*.

The cleavage of Aplysia, so far as followed by Blochmann, resembles very closely that of Umbrella. If we interpret the cleavage as Heymons has that of Umbrella, we find that here also the posterior and smaller cell of the two-cell stage divides first; the mesoderm quadrant *D* (labelled *c* by Blochmann) also precedes in some instances in the later divisions. The yolk, however, is confined mainly to two of the macromeres, *A* and *B*, instead of being lodged principally in three, *A*, *B*, and *C*, as it is in Umbrella.

All attempts to reconcile the cleavage of Chiton, as described by Kowalevsky ('83) and Metcalf ('93), with the alternation of spirals shown in other forms of Molluscan cleavage are involved in serious difficulties. Unfortunately neither author figures nuclei or spindles, with the exception of one figure in each paper. The relationship of cells and the direction of the spiral is also explicitly stated in certain cases to be a *matter of conjecture* on their part. Therefore it does not seem profitable for me to add to their conjectures others of my own. It is however possible to force upon the cleavage of Chiton, as figured by these authors, an interpretation which causes it to accord with the spiral type of cleavage, but this interpretation meets a serious obstacle in the sixteen-cell stage, though it does not violate the relationships of cells in the few cases where these authors have indicated relationships by nuclear conditions (Kowalevsky, Plate I. Fig. 7, Metcalf, Plate XV. Fig. xvii.).

I do not wish to commit myself to the view that Chiton conforms to that type, for, as Metcalf has suggested, "most of the divisions are of the radial type." The distribution of the yolk in the blastomeres is also suggestive of the radial type. There are, however in Kowalevsky's figures (Plate I. Figs. 4–13) many suggestions of spiral cleavage. In Metcalf's figures, on the other hand, "individual variations in the shape of the blastomeres are not preserved. The figures show what may be called the typical condition." Thus most of the evidence of spiral cleavage, if such exists, must be eliminated from his figures.

The cleavage of Discocœlis conforms to the spiral type and the alternation of spirals, with the possible exception of the spiral of 7.15–7.16, to which reference was made in my former paper ('94, p. 196). It is necessary to relabel Figure 6 of Lang's Tafel XXXV., in order to reconcile it with the apical views which he has given of the same stage, and also with the principle of alternation.

The conformity of the cleavage of Nereis to the law of alternation of spirals is perfect, as is demonstrated by the application of the uniform system of naming spirals in the above table. Even in those cleavages designated as equatorial or meridional, traces of the spiral characteristic of the generation can often be detected.

The Nereis table is marked by the abrupt termination of the spiral

CLEAVAGE OF DISCOCŒLIS.

Lang ('85).

LANG'S NOMENCLATURE.		Generation.	Number of Cells.	REVISED NOMENCLATURE.	
Spirals.	Cells.			Cells.	Spirals
Left.	$\begin{matrix} ae_1 \\ ae_6 \end{matrix} > ae_1$	VII.	44	$a^{6.8} < \begin{matrix} a^{7.16} \\ a^{7.15} \end{matrix}$	Right, contradicts law of alternation.
——	$\begin{matrix} ae_5 \\ ae_3 \end{matrix} > ae_3$		40	$a^{6.7} < \begin{matrix} a^{7.14} \\ a^{7.13} \end{matrix}$	Left?
——	$\begin{matrix} am_3 \\ am_1 \end{matrix} > am_1$		52	$a^{6.4} < \begin{matrix} a^{7.8} \\ a^{7.7} \end{matrix}$	Left.
Right.	$\begin{matrix} a \\ auen \end{matrix} > a$		32	$A^{6.1} < \begin{matrix} A^{7.2} \\ a^{7.1} \end{matrix}$	Left.
Left.	$\begin{matrix} ae_1 \\ ae_3 \end{matrix} > ae_1$	VI.	28	$a^{5.4} < \begin{matrix} a^{\cdot 8} \\ a^{6.7} \end{matrix}$	Right.
Left.	$\begin{matrix} ae_2 \\ ae_4 \end{matrix} > ae_2$		36	$a^{5.3} < \begin{matrix} a^{6.6} \\ a^{6.5} \end{matrix}$	Right.
Right.	$\begin{matrix} am_1 \\ am_2 \end{matrix} > am_1$		24	$a^{5.2} < \begin{matrix} a^{6.4} \\ a^{6.3} \end{matrix}$	Right.
Right.	$\begin{matrix} a_2m_1 \\ a \end{matrix} > a$		20	$A^{5.1} < \begin{matrix} a^{6.2} \\ A^{6.1} \end{matrix}$	Right.
Right.	$\begin{matrix} ae_1 \\ ae_2 \end{matrix} > ae_1$	V.	16	$a^{4.2} < \begin{matrix} a^{5.4} \\ a^{5.3} \end{matrix}$	Left.
Left.	$\begin{matrix} am_1 \\ a \end{matrix} > a$		12	$A^{4.1} < \begin{matrix} a^{5.2} \\ A^{5.1} \end{matrix}$	Left.
Right.	$\begin{matrix} ae_1 \\ a \end{matrix} > a$	IV.	8	$A < \begin{matrix} a^{4.2} \\ A^{4.1} \end{matrix}$	Right.
Left.	$a, b, c, d.$	III.	4	$A, B, C, D.$	Left.
		II.	2	$\overline{AB}, \overline{CD}.$	

CLEAVAGE OF NEREIS.

Wilson ('92).

Wilson's Nomenclature.		Generation.	Number of Cells.	Revised Nomenclature.	
Spirals.	Cells.			Cells.	Spirals.
Left.	$\begin{matrix}a^{1.3}\\ a^{1}\end{matrix} > a^{1}$	VII.	36	$a^{6.8} < \begin{matrix}a^{7.16}\\ a^{7.15}\end{matrix}$	Left, *c* and *d* precede.
Left.	$\begin{matrix}a^{1.2.2}\\ a^{1.2.1}\end{matrix} > a^{1.2}$		——	$a^{6.7} < \begin{matrix}a^{7.14}\\ a^{7.13}\end{matrix}$	Left.
Horizontal.	$\begin{matrix}a^{1.1.2.2}\\ a^{1.1.2.1}\end{matrix} > a^{1.1.2}$		42–58	$a^{6.6} < \begin{matrix}a^{7.12}\\ a^{7.11}\end{matrix}$	Equatorial.
Vertical.	$\begin{matrix}a^{1.1.1.2}\\ a^{1.1.1.1}\end{matrix} > a^{1.1.1}$		42–58	$a^{6.5} < \begin{matrix}a^{7.10}\\ a^{7.9}\end{matrix}$	Meridional.
——	$\begin{matrix}X\\ x^{2}\end{matrix} > X$		37 +	$d^{6.4} < \begin{matrix}d^{7.8}\\ d^{7.7}\end{matrix}$	Left, *d* precedes.
Horizontal.	$> a^{2.2}$		——	$a^{6.3} < \begin{matrix}a^{7.6}\\ a^{7.5}\end{matrix}$	Equatorial.
Horizontal.	$> a^{3}$		——	$a^{6.2} < \begin{matrix}a^{7.4}\\ a^{7.3}\end{matrix}$	Equatorial.
Left.	$\begin{matrix}M\\ D\end{matrix} > D$		38 +	$D^{6.1} < \begin{matrix}d^{7.2}=M\\ D^{7.1}\end{matrix}$	Left, *D* precedes.
Left.	$\begin{matrix}a^{1}\\ a^{1\,2}\end{matrix} > a^{1}$	VI.	20	$a^{5.4} < \begin{matrix}a^{6.8}\\ a^{6.7}\end{matrix}$	Right.
Meridional.	$\begin{matrix}a^{1.1.2}\\ a^{1.1.1}\end{matrix} > a^{1.1}$		29	$a^{5.3} < \begin{matrix}a^{6.6}\\ a^{6.5}\end{matrix}$	Right, approaching meridional.
Left.	$\begin{matrix}a^{2.2}\\ a^{2.1}\end{matrix} > a^{2}$		23–32	$a^{5.2} < \begin{matrix}a^{6.4}\\ a^{6.3}\end{matrix}$	Right, *d* precedes.
Right.	$\begin{matrix}a^{3}\\ A\end{matrix} > A$		23–29	$A^{5.1} < \begin{matrix}a^{6.2}\\ A^{6.1}\end{matrix}$	Right, *C* and *D* precede.
Right.	$\begin{matrix}a^{1}\\ a^{1.1}\end{matrix} > a^{1}$	V.	16	$a^{4.2} < \begin{matrix}a^{5.4}\\ a^{5.3}\end{matrix}$	Left.
Left.	$\begin{matrix}a^{2}\\ A\end{matrix} > A$		16	$A^{4.1} < \begin{matrix}a^{5.2}\\ A^{5.1}\end{matrix}$	Left, *D* precedes.
Right.	$\begin{matrix}a^{1}\\ A\end{matrix} > A$	IV.	8	$A < \begin{matrix}a^{4.2}\\ A^{4.1}\end{matrix}$	Right, *C* and *D* precede.
	A, B, C, D.	III.	4	*A, B, C, D.*	Left.
	$\overline{AB}$, $\overline{CD}$.	II.	2	$\overline{AB}$, $\overline{CD}$.	

CLEAVAGE OF UMBRELLA.

Heymons ('93).

Heymons's Nomenclature.		Generation.	Number of Cells.	Revised Nomenclature.	
Spirals.	Cells.			Cells.	Spirals.
—	$\genfrac{}{}{0pt}{}{a''_{1.3}}{a''_1} > a''_1$	IX.	75	$a^{8.14} < \genfrac{}{}{0pt}{}{a^{9.28}}{a^{9.27}}$	Meridional.
Right.	$\genfrac{}{}{0pt}{}{a''_{1.2}}{a''_{1.2.1}} > a''_{1.2}$		79	$a^{8.13} < \genfrac{}{}{0pt}{}{a^{9.26}}{a^{9.25}}$	Left.
—	$\genfrac{}{}{0pt}{}{a''_1}{a''_{1.2}} > a''_1$	VIII.	51	$a^{7.7} < \genfrac{}{}{0pt}{}{a^{8.14}}{a^{8.13}}$	Equatorial, trace of right.
—	$\genfrac{}{}{0pt}{}{a''}{a''_3} > a''$		55	$a^{7.6} < \genfrac{}{}{0pt}{}{a^{8.12}}{a^{8.11}}$	Equatorial.
Left, *a* and *b*. Merid., *c* and *d*.	$\genfrac{}{}{0pt}{}{a'''}{a'''_2} > a'''$		40–59	$a^{7.4} < \genfrac{}{}{0pt}{}{a^{8.8}}{a^{8.7}}$	Right, *a* and *b*. Merid., *c* and *d*.
Meridional.	$\genfrac{}{}{0pt}{}{a'''_1}{a'''_{1.1}} > a'''_1$		67	$a^{7.3} < \genfrac{}{}{0pt}{}{a^{8.6}}{a^{8.5}}$	Meridional, *a* and *b* precede.
—	$\genfrac{}{}{0pt}{}{A''}{A} > A$		91 +	$A^{7.1} < \genfrac{}{}{0pt}{}{A^{8.2}}{A^{8.1}}$	Bilateral, trace of right.
Like Nereis.	Not figured.	VII.	91 +	$a^{6.8} < \genfrac{}{}{0pt}{}{a^{7.16}}{a^{7.15}}$	Left.
Right.	$\genfrac{}{}{0pt}{}{a'_2}{a'_{2.1}} > a'_2$		71	$a^{6.7} < \genfrac{}{}{0pt}{}{a^{7.14}}{a^{7.13}}$	Left.
Left.	$\genfrac{}{}{0pt}{}{a_1''{}_{.1}}{a_1''} > a''_1$		33	$a^{6.4} < \genfrac{}{}{0pt}{}{a^{7.8}}{a^{7.7}}$	Left, *b* and *c* precede.
Left.	$\genfrac{}{}{0pt}{}{a''}{a''_2} > a''$		37	$a^{6.3} < \genfrac{}{}{0pt}{}{a^{7.6}}{a^{7.5}}$	Left, *b* and *d* precede.
Left.	$\genfrac{}{}{0pt}{}{a'''}{a'''_1} > a'''$		29	$a^{6.2} < \genfrac{}{}{0pt}{}{a^{7.4}}{a^{7.3}}$	Equatorial, trace of left.
Left.	$\genfrac{}{}{0pt}{}{A'}{A} > A$		25–47	$A^{6.1} < \genfrac{}{}{0pt}{}{A^{7.2}}{A^{7.1}}$	Left, *D* precedes.
Left.	$\genfrac{}{}{0pt}{}{a'}{a'_2} > a'$	VI.	44	$a^{5.4} < \genfrac{}{}{0pt}{}{a^{6.8}}{a^{6.7}}$	Right.
Right.	$\genfrac{}{}{0pt}{}{a'_1}{a'_{1.1}} > a_1'$		65	$a^{5.3} < \genfrac{}{}{0pt}{}{a^{6.6}}{a^{6.5}}$	Equatorial, approaching bilateral, *c* and *d* precede.
Right.	$\genfrac{}{}{0pt}{}{a''_1}{a''} > a''$		24	$a^{5.2} < \genfrac{}{}{0pt}{}{a^{6.4}}{a^{6.3}}$	Right.
Right.	$\genfrac{}{}{0pt}{}{a'''}{A} > A$		16	$A^{5.1} < \genfrac{}{}{0pt}{}{a^{6.2}}{A^{6.1}}$	Right, *C* and *D* precede.
Right.	$\genfrac{}{}{0pt}{}{a'}{a'_1} > a'$	V.	20	$a^{4.2} < \genfrac{}{}{0pt}{}{a^{5.4}}{a^{5.3}}$	Left.
Left.	$\genfrac{}{}{0pt}{}{a''}{A} > A$		12	$A^{4.1} < \genfrac{}{}{0pt}{}{a^{5.2}}{A^{5.1}}$	Left.
Right.	$\genfrac{}{}{0pt}{}{a'}{A} > A$	IV.	8	$A < \genfrac{}{}{0pt}{}{a^{4.2}}{A^{4.1}}$	Right.
Left.	*A*, *B*, *C*, *D*.	III.	4	*A*, *B*, *C*, *D*.	Left.
	$\overline{AB}$, $\overline{CD}$.	II.	2	$\overline{AB}$, $\overline{CD}$.	$\overline{CD}$ precedes.

period in the seventh generation. The cleavages of the following generations are meridional or equatorial, and belong to the bilateral period. Another noticeable feature is the general precedence of the mesoderm-producing quadrant *d* in the cleavages of the various quartets.

The bilateral period in the cleavage of Umbrella is not so sharply marked off from the spiral period as it is in Nereis; in Umbrella spiral cleavage occurs as late as the ninth generation. In Nereis it ceases in the seventh. The quartets 5.3 and 6.2 *in both forms* are the ones in which the spiral character of the division first gives way to the meridional and equatorial cleavage, — characteristic of the bilateral period.

The cleavage of Umbrella, like that of Nereis, presents no contradictions to the law of alternation of spirals. This striking agreement of Nereis, Umbrella, and Limax must far outweigh any seeming contradiction arising in the work of the earlier writers upon spiral cleavage. It is only necessary to apply the proposed system of nomenclature to the careful work of Wilson and Heymons to make clear at once that the alternation defended holds good. The system of nomenclature employed in this paper facilitates the demonstration of the alternation of spirals in successive generations of cells; but the alternation itself is a factor independent of mere names. It is the fundamental basis of the so called "spiral type" of cleavage. A recognition of this fact might well be embodied in nomenclature, and *alternating cleavage* substituted for the ambiguous and misleading term "spiral cleavage."

D. The Mesoderm.

In the forty-four-cell stage, at which the discussion of the cleavage of Limax was dropped, the germ layers are already differentiated. The quartets 7.3, 7.4, 6.3, 7.7, 7.8, 6.5, 6.6, 6.7, 6.8, are pure *ectoderm*, the quartet 7.1 and three fourths of the quartet 7.2, viz. $a^{7.2}$, $b^{7.2}$, and $c^{7.2}$, are *entoderm*, while $d^{7.2}$ is the sole representative of the *middle germ layer*.

The seven entoderm cells and their progeny come to lie in the region of the blastopore, and with the invagination are carried in to form the lining of the archenteron. The primary mesoblast divides bilaterally, i. e. in the median plane of the embryo, shortly after the forty-four-cell stage. The two mesoblasts retain a slight connection with the exterior, and at the ninety-cell stage have each divided transversely, the peripheral and posterior pair of cells are the *smaller*, and retain a slight connection with the exterior. The next division occurs in the anterior pair. The cells of the mesoderm continue to multiply until there are formed upon either side of the median line, extending forward from the posterior lip of the blastopore, two lateral bands of mesodermal elements. These bands are shown in longitudinal section in Figures 48, 49, and 50 (Plate VII.); in transverse section, in Figures 45 and 46. In Figure 48 the band consists of five cells, the posterior one of which in this stage is the largest. The band is somewhat curved, so that the anterior ends diverge from the more closely approximated posterior cells. In Figure 50 the lateral band contains six cells, the two at the posterior end being in a mitotic state. The position of their spindles is significant of the manner in which the bands have arisen, viz. by proliferation anteriorly from the posterior cell and its products. The "pole cell" as such is not sharply distinguished from the rest of the band by its size, as it is in Umbrella and Nereis. The position of the nuclei of two of the cells is suggestive of a division in a plane coinciding with the axis of the band. I have however never found a spindle in a plane perpendicular to that axis, though spindles parallel with the axis of the band are frequently found.

I have seen no evidence whatever that any of the cells of these mesodermal bands are derived from any other source than the primitive mesoblast, $d^{7.2}$. It is of course impossible to prove that none of the cells can have come from the external layers, either by migration inward, or by the division of a superficial cell in a plane parallel to the surface; but in the absence of any evidence that this does take place, and with such proof as Figure 50 (Plate VII.) gives, it seems not unreasonable to hold that the entire mesoderm is derived from the one cell, $d^{7\,2}$. A comparison of the origin and development of the mesoderm in Nereis and Limax shows a precisely identical origin in the two forms. In Nereis, however, the mesoderm shares in the generally much more accelerated development, so that, although it appears at about the same cell stage in both forms, the relative number of mesoderm cells in Nereis in the later stages is much greater than in a corresponding stage of Limax. The accelerated division of the mesodermal quadrant (*D*) in the cleavages of the different quartets, as noted by Wilson and shown in the table of the cleavage of Nereis, may be a manifestation of this same accelerating force. I have not been able to find any trace of such a differentiation in the cleavage of the quadrants in Limax, where there are no early appearing larval organs and little acceleration in the development of the mesoderm. On the other hand, Lillie ('93) has been able to establish in Unio the same tendency of the quadrant *D* to precede in division; but in Unio again there is a very early development of larval organs. In Umbrella this tendency is not so marked, and here definite protoblasts are not distinguishable as early as they are in Nereis.

The later history of the mesoderm will be discussed in connection with the subject of gastrulation and the fate of the blastopore. There is never developed within these mesoblast bands, at any period of their history, a lumen, either such as Erlanger has described for Bythinia ('92) and for Capulus ('92^{a}), or of any other kind. The bands later lose their distinctness and break up into loose mesenchyma in which it is no longer possible to distinguish pole cells. The mesenchyma cells make their way between the ectoderm and entoderm layers, and by their multiplication and accumulation in different regions exercise a profound influence upon the form of the embryo. The obliteration of the mesoblast bands by this process renders the determination of the relation of the axes of these bands to the axes of the adult very difficult.

Inasmuch as both Erlanger ('91) and Heymons ('93) have recently given very full and satisfactory reviews of the conflicting literature on the origin of the middle germ layer in the Mollusca, it hardly seems necessary for me to go over the same ground. It will suffice in passing to call attention to the identity of my results, as to the origin of this layer in Limax, with those of Heymons ('93) on Umbrella, Lillie ('93) on Unio, Conklin ('91, '92) on Crepidula, Blochmann ('81) on Neritina, and Rabl ('79) on Planorbis, making allowance of course for possible differences in the case of Planorbis due to reversed cleavage. It seems very probable that the mesoderm may have a similar origin, i. e. from $d^{7.2}$, in the Pteropods (Fol '75 and Knipowitsch '91), in Aplysia (Blochmann '83), and in Fulgur (McMurrich '86).

E. Theoretical Considerations.

The question as to the relation existing between the method of formation of the mesoderm described by Erlanger for Paludina and Bythinia, and the type presented in Umbrella, can find its satisfactory answer only in an examination of these first named forms from the cytogenetic standpoint. As the matter stands now, we are compelled to deny the morphological significance of the precise method of the origin of the middle layer, if we maintain its homology even within the group of the Mollusca.

The method of origin of the mesoderm in Cyclas, as well as the cleavage according to Stauffacher's description ('93), presents the material for an interesting comparison with that of its near ally, Unio. In the latter case the cleavage is spiral (Lillie '93), and the mesoderm (adult) comes, as in other cases of spiral cleavage, from $d^{7.2}$. In Cyclas, on the other hand, is found a unique type of cleavage, mesenchyma appearing early, possibly at the seventh generation ; but the "mesoderm," as distinguished from the mesenchyma cells, appears much later, and is not separated from the entoderm *before* its bilateral division. Such a case as this shakes one's faith in homologies based on forms of cleavage or cell lineage. Indeed, it seems impossible in the face of these conflicting results to assign to these phases of embryonic development any definite phyllogenetic significance. On the other hand, the identity of the cleavage processes among certain of the Mollusca (Umbrella, Unio, Crepidula, Neritina, and Limax), and the similarity of cleavage in these to that of an entirely different group of animals, viz. the Annelids, are phenomena not easily banished from the thought. They must have some significance, some common cause. To my mind the appeal to simple mechanical principles as an explanation of the phenomenon which, broadly speaking, we call the spiral, or alternating, type of cleavage, affords little satisfaction. If the principle of "the resumption of the spherical form," or that of "minimal surfaces of contact," prevails in one egg, why should it not in all eggs? We find the spiral type occurring in eggs with no, with little, or with much yolk, and the yolk, when present, variously distributed in the blastomeres; yet the spirals occur with absolute certainty and in a definite manner. Other eggs, presenting apparently the same mechanical conditions, cleave in accordance with an entirely different system, radial or bilateral, in both of which adaptations to mutual pressure may occur without a distinct spiral.

We can find a satisfactory explanation of the bilateral type of cleavage. It is simply an accelerated victory of a force which sooner or later dominates every developing egg of the Bilateria. Thus it is that the spiral type itself gives way to the bilateral, as Wilson has so well shown in Nereis.

It must be evident to all that the spiral type is very prevalent among the Trochozoa, i. e. among forms in which a free-swimming larva is early developed.

Thus, in Nereis at the thirty-eight-cell stage, not only are the germ layers completely differentiated, but most of the individual blastomeres are set apart as protoblasts from which definite organs or parts of the adult body are soon to arise. This occurs about five hours after fertilization, and at ten to eleven hours after that event the larva begins to rotate. Here we have a complete histological differentiation, while as yet only a comparatively small number of cells are present. Whether or not there exists any causal nexus between precocious development and the spiral type of cleavage, is a question upon which experimental embryology may be destined to throw some light; as yet experimentation has been confined to eggs having the radial or bilateral form of cleavage.

The three forms of cleavage, radial, spiral, and bilateral, are undoubtedly connected. Wilson ('93, p. 600) has suggested that the spiral type is a modification of the radial, and owes its peculiarities to mechanical conditions. I would also suggest that spiral and *bilateral* types are very intimately connected. The spirally cleaving egg is essentially bilateral from the time that the first cleavage plane appears, and an inspection of the tables of the cleavage of Nereis, Umbrella, and Limax shows that the embryo becomes predominantly bilateral as the spiral cleavage fades out. In Nereis the transition from the spiral to the bilateral period is abrupt; in Umbrella and Limax the two periods overlap during several generations. The cleavages which succeed those of the spiral type are meridional and equatorial, and I believe are to be referred to the bilateral rather than the radial type; indeed, in some cases, as in the division of 5.3 and 7.1 in Umbrella, the division approaches very closely the typical bilateral cleavage of the tunicate egg, i. e. is symmetrical with reference to the median plane of the embryo. Wilson ('92, p. 391) has referred the meridional cleavage of 5.3 to the *radial* type. In Nereis this quartet divides before the mesoderm appears; in Umbrella and Limax after it appears. When, however, in Nereis the quartet 7.15 divides equatorially *after* the mesoderm is formed, Wilson refers this division to the bilaterai type. It seems to me that all these equatorial and meridional cleavages succeeding the spiral divisions both before and after the mesoblast appears must be referred to the bilateral period of the embryo and to the bilateral type of cleavage.

The precise agreement of Umbrella, Nereis, and Limax in these first bilateral cleavages is evidently something more than mere accident. The meridional character of the division in two of the cases (Limax and Nereis) suggests the possibility of similar mechanical conditions. But if all the conditions in the two cases are compared more closely, it becomes clear that there are important differences. The cleavage in question takes place in Nereis at the twenty-nine-cell stage, in Limax at the forty-four, and a comparison of Figure 39 or Figure 41 (Plate

VI.) with Wilson's Plate XV. Fig. 23 shows at once the great difference in the shape of the egg, and the mechanical environment of the cells under consideration. The evidence in this case, therefore, seems to point to some other force than that of mechanical condition as the determining cause of this remarkable agreement.

The intimate association of the spiral and bilateral types of cleavage, and also the prevalence of spiral cleavage in those animals possessing precociously developed larval forms, in which bilateral symmetry and histological differentiation are early impressed upon the cleaving ovum, suggest that the cause of spiral cleavage does not lie entirely in the external mechanical environment of the cells, but is, in part at least, to be referred to the same "morphogenic force" which produces the bilateral symmetry of the embryo and the adult. That the ultimate fate of cells exercises a profound influence upon their cleavage is well shown in the precocious cleavage of the mesoderm quadrant in Nereis and Unio, and of the teloblasts of the larval excretory organs in Umbrella. It may be that in like manner spiral cleavage itself is but a manifestation of precocious development of the organism as a whole.

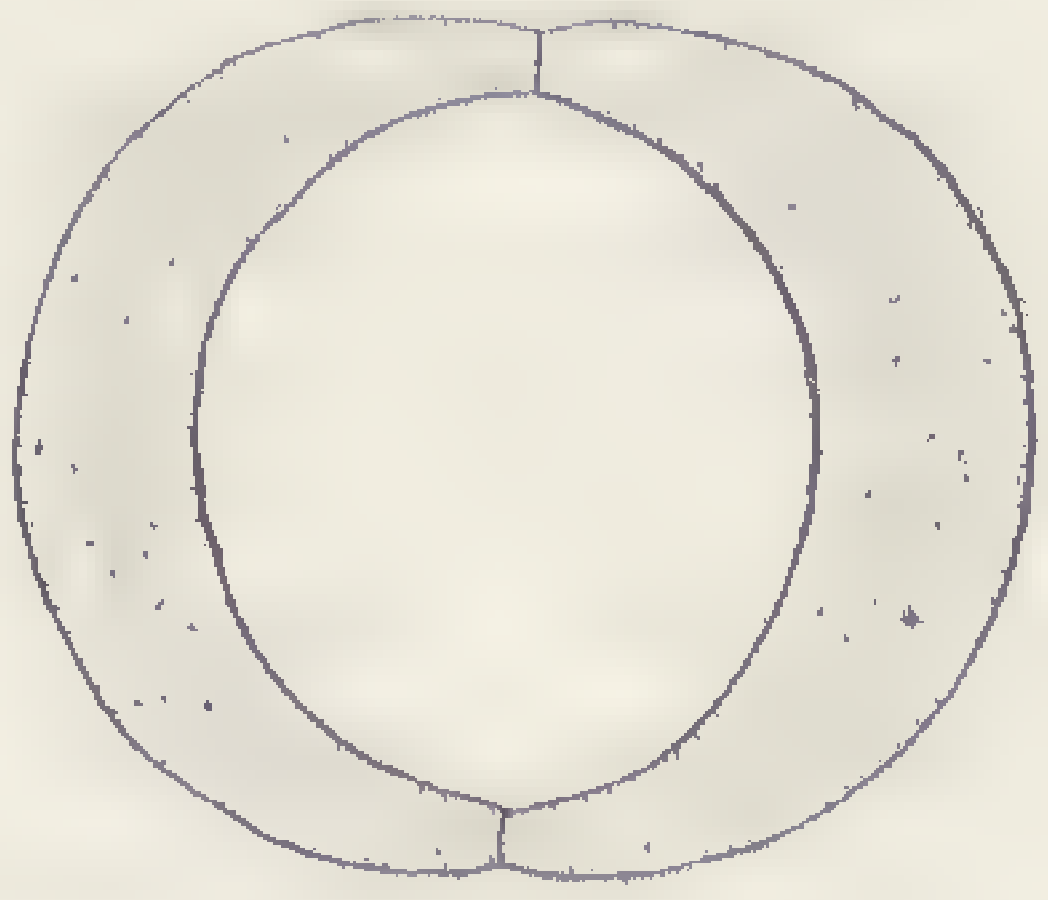
It is also difficult to explain the *alternation* of spirals by the mechanical conditions attending their formation. A glance at the tables of cleavage which I have given will quickly suggest that, although we have the same spiral in a given division of any generation in *all the eggs* having spiral cleavage, the conditions under which the spiral is formed in the eggs of different animals are by no means identical. The chronological sequence of the division of quartets in different eggs is not the same; neither is the distribution of the yolk, either in quantity or quality. The spirals however are always identical wherever they occur. These external mechanical conditions have doubtless a profound influence, but are they the only or the prevailing ones? If we predicate this, we must maintain that the resultants of these variously combined mechanical influences are identical in all cases of identical spirals. Be the *cause of the spiral* what it may, the internal conditions of nuclear division seem to be correlated with the *alternation* in direction in successive generations. In an unimpeded field of action, the division and subsequent equal migration of the two daughter centrosomes would necessarily produce a series of cell divisions at right angles to one another. This element is doubtless one of the factors in that field of complex activities, the cleaving ovum.

F. The Cleavage Cavity.

1. *In Limax.*

After the blastomeres have reached the widely divergent state seen in Plate I. Fig. 14, they begin to flatten against each other, gradually losing their individual spherical contour and assuming a hemispherical shape. This process occupied, in a case recorded, about an hour, and was comparatively more rapid in the latter part. It results in the approximate restoration of the egg to the form of a single sphere. The superficial region of contact of the two cells appears in the living egg as a somewhat irregular line in the now almost obliterated furrow. Very soon after this process is completed there appear along this line lenticular or irregular spaces, devoid of the granular structure of the protoplasm, and apparently filled with a clear fluid. Deeper focusing reveals the fact that the centre of the apposed faces of the blastomeres is occupied by a slight cavity, wedge-shaped toward the vegetative pole, and broader and rounded toward the animal pole. This cavity gradually increases in size, the minor lenticular spaces increase also, and contiguous ones may be seen to coalesce. Finally, as the central cavity increases more and more, and approaches the periphery of the facet of contact, the lenticular spaces themselves disappear, probably contributing their contents to the encroaching central cavity. The latter now presents the form of a broadly lenticular clear space extending from the animal to the vegetative pole of the egg, and symmetrically developed with reference to these poles. The two cells are thus almost completely separated from each other by the fluid filling the cavity, as will be seen in the accompanying Figure E, giving an optical section in the plane of the equator of a two-cell stage of Limax agrestis, showing a cleavage cavity. They remain in intimate connection, however, at the peripheral margin, but this margin of union is in some cases reduced to a very thin layer of protoplasm. There is apparently no difference in the extent of the union at the two poles. The growth of the cavity results in an appreciable increase in the volume of the egg, and its contour, as well as that of the cavity itself, is suggestive of the high state of tension existing in the egg as a result of this increase in volume. In extreme cases, as in Plate V. Fig. 34, and in Figure E, the

Figure E.



cavity may attain a volume equivalent to one half or two thirds that of the undivided ovum. Throughout its whole history, from its inception to its culmination, both in the living egg and in preserved material, the cavity is always sharply marked off from the protoplasm of both cells. This is true, no matter what may be the point of view from which the egg is observed. Of course the boundaries of the cavity are indistinct where they lie oblique to the optical axis of the microscope, but there is always one focal plane at which the limiting "membrane" of the bounding cells sharply and distinctly separates the protoplasm from the fluid contents of the cavity, and moreover there is no trace whatever of any shading off of the protoplasm toward the cavity such as Stauffacher ('93) describes in Cyclas. We are therefore compelled to conclude that the cavity is distinctly intercellular.

The maximum development of the cavity is followed by a forcible expulsion of its contents. This takes place suddenly, and the elimination of the fluid may be total, or only partial. After a total elimination the egg resumes its original size, and tends to take again the spherical form. In case of a partial expulsion the cavity retains its polar diameter, but the antero-posterior diameter is reduced (Plate I. Figs. 5, 6). In the egg represented in Figure E, the spindles leading to the third generation were present when the cavity of the two-cell stage had reached its maximum. It often happens that two or even more expulsions of the liquid contents of the cavity occur between the two- and the four-cell stages. In the series represented in Plate I. Figs. 1–7, drawn from the same egg kept under continuous observation, the two blastomeres had attained the maximum divergent or rounded condition at 11.30 A. M. At 12.35 P. M. they had reached the flattened or coalescent condition (Figs. 1, 2), and at 12.45 P. M. the central cavity had appeared (Fig. 2). This increased in size (Figs. 3–5), reaching a maximum at 1.30 P. M., when a partial expulsion occurred (Fig. 6). After this expulsion the nuclei could no longer be seen distinctly in the living egg. The cavity again increased in volume (Figs. 6, 7), and at 2.02 P. M. a second and total elimination took place coincidently with the division into four cells; this was accomplished, i. e. the furrows had reached the vegetative pole, at 2.05 P. M.

It is not always possible to determine the point at which the fluid contents are ejected. When a sudden reduction in the size of the cavity is noticed, there is sometimes visible in the albumen adjacent to the cleavage furrow a small sphere of transparent matter differing in its refractive index from the surrounding albumen into which it very quickly merges. When, however, the reduction in the size of the cavity is gradually accomplished, occupying in one instance recorded about five minutes, no trace of the extruded liquid is visible. I have observed in the two-cell stage the expulsion of the liquid at both animal and vegetative poles, but never at both poles of an egg at the same time. Warneck ('50) and Fol ('80) both state that the contents of the cavity are expelled at the vegetative pole. This is certainly by no means constant, and I am inclined to believe that in a majority of cases, especially in the later stages, the elimination takes place at the animal pole of the egg.

This ephemeral cleavage cavity is not confined in Limax to the two-cell stage, but is equally prominent in the stages immediately following. The passage of the egg from the two- to the four-cell stage may be accompanied by an incomplete elimination of the contents, for I have often observed cases where a small cavity persists throughout the progress of this cleavage.

Figures 8–13 (Plate I.) show the history of the cleavage cavity in a different egg from the one observed during the two- to four-cell stage. At 3.15 P. M. there was no trace of any cleavage cavity, and the second cleavage furrow had almost reached the vegetative pole. Half an hour later the characteristic four-cell condition had been reached (Fig. 8), and in ten minutes more a cleavage cavity of considerable volume was developed in the vertical axis of the egg. This continued to increase in size until 4.45 P. M. (Figs. 9–11), when a total expulsion of the contents occurred, occupying not more than thirty seconds (Fig. 12). The nuclei at this period were at the amphiaster stage. Within fifteen minutes a new cavity had appeared in the now elongated vertical axis of the egg. This cavity was at first very narrow and extended almost from pole to pole. It increased slowly in volume, but was not wholly obliterated at the division into eight cells, which occurred at 5.38–5.45 P. M. (Fig. 13). It is not at all unusual to see the total elimination of the contents of the cavity at the division into eight cells, but the occurrence is not constant. The configuration of the cavity of the four-cell stage as viewed from the animal pole is shown in Plate II. Fig. 17. It is almost rhomboidal in outline; the angles lie at the cleavage planes, and the sides are curved with the convexity next the cavity. It is probable that a partial expulsion, or perhaps a total one, has already occurred, for the cavity was not very large and the nuclei were in the early phase of metakinesis when the egg was killed. When the cavity is at its maximum it assumes very nearly a spherical shape, i. e. the bounding cells are concave toward the cavity, and they present more nearly the character of a wall of uniform thickness (Fig. E, p. 81). No case has come under my observation where a nucleus projects into the cavity, as Stauffacher ('93) figures it in his Tafel XIII. Fig. 19 *a*.

An interesting condition is found in Plate II. Fig. 16, in which the second cleavage furrow is almost completed. The cavity appears to have been divided into two parts by the recent cleavage furrow, and now consists of two large lenticular spaces, one between the cells *A* and *D*, the other between *B* and *C*, i. e. both spaces are in the first cleavage furrow. The first appears to lie mainly in the cell *A*, but this is due to the fact that *A* lies slightly above and upon *D*. The cavity between *B* and *C* has several secondary contributory spaces lying superficially to it in the furrow at the animal pole.

The cavity of the eight-cell and later stages differs from that of the two-cell stage in that it is situated nearer the animal than the vegetative pole of the egg. This is correlated with the size of the two quartets of the fourth generation, Plate III. Figs. 20 and 21, and may be the occasion of the frequent escape of the fluid contents at the animal pole.

It is not necessary to follow in detail the phenomena which attend the further history of the cleavage cavity, as it would be in the main a repetition of the description of that of the earlier stages. I shall merely call attention to certain features of the cavity which are of especial interest.

An examination of a large number of eggs in the living state, as well as killed and hardened material studied both *in toto* and in sections, has led me to the conclusion that this ephemeral and recurrent phase of the cleavage cavity or blastocœl continues until a late stage, even to the period of gastrulation. That its appearance is not due to a pathological condition of the embryo is shown by the prevalence of the same phenomenon in eggs collected in the natural environment of the slug, as well as by the development of normal embryos from vacuolated eggs. It may be that confinement conduces to the presence of the ephemeral cavity in its various forms, but I have no direct evidence that such is the case.

Eggs presenting the maximum development of the cleavage cavity in the later stages are with great difficulty freed intact from their envelopes and require especial care in the subsequent treatment with reagents. On Plate V. (Figs. 33 and 34) is figured an egg of twenty-four cells with a well developed cleavage cavity. The nuclei are all in a quiescent state, and the cells form a wall of such uniform thickness that it was only after repeated trials that the vegetative pole of the egg could be determined. The cavity is so large that the facets of contact are very narrow, and the individual cells are in optical section somewhat lozenge-shaped. The cell $a^{6.2}$ (Fig. 34) presents a curious bud-like process extending into the central cavity, and the superficial extent of the cell is somewhat less than that of the other members of the same quartet. This process suggests the mesenchyma cells which Stauffacher ('93) figures in his Tafel XIV. Figg. 25 *a* and 25 *c*, but in this projection there is not the least trace of any nuclear structure, and it is probably a mere amœboid outgrowth of no permanent significance.

A comparison of the computed volumes of the whole egg, of its cavity, and of the protoplasmic portion, with the volume of another egg (Plate IV. Fig. 27) of the same stage but having no cavity, brings out the following results. The whole egg has 429 units of volume, of which 188 represent that of the cavity, 241 that of the blastula wall, while the twenty-four-cell stage of average size (Fig. 27) has a volume of only 126 units. These figures assume the perfect sphericity of the objects measured, and are therefore only approximately correct; still they show that the first egg, though a large one, is within the limits of variation in size, and that the cavity is larger than the average egg, but not so voluminous as the substance of the egg which contains it. It is also suggested, in view of the large size of the egg, that the cavity has not been developed to any great extent at the expense of the volume of the protoplasm of the egg. There can be no question that this egg presents the condition of a typical "blastula" with a typical "cleavage cavity" or blastocœl. Indeed, Rabl could not have found for Haeckel and his Gastræa Theory a better illustration among mollusks of the "morula" and "blastula" stages than these two twenty-four-cell stages (Plate IV. Fig. 27 and Plate V. Fig. 34); for the first contains no cavity whatever, and the latter has its cells arranged in a single layer about a cavity. On the other hand, if we accept the limitation set upon our usage of the term cleavage cavity by Stauffacher in his recent paper ('93), we shall be compelled, in view of the fact that the cavity is sooner or later entirely eliminated, to call this beautiful example of a cleavage cavity simply "ein heller Raum."

It is difficult to establish any regularity or uniformity in the sequence of the phases of the cavity in these later stages of cleavage. When we examine other eggs in the twenty-four-cell stage we meet with different and by no means constant conditions. The twenty-four-cell stage represented in Plate IV. Fig. 31, shows no trace whatever of a cavity; while Figure 28, also a twenty-four-cell stage, shows at the animal pole a number of lacunæ or intercellular vacuoles between the cells of the apical quartet, $a^{5.4} — d^{5.4}$ and their neighbors. An especially large vacuole is formed immediately at the animal pole.

Inasmuch as the vacuolation of the animal half of the egg is an important and very prevalent occurrence in the later stages in the cleavage of Limax it deserves a detailed description. In surface view these cavities are seen to be arranged in general along the line of the cell boundaries, which they obscure to such an extent that the superficial margins of the facets of contact are detected only by careful focusing upon the immediate surface of the egg properly illuminated. As soon as the plane of the focus is lowered toward the level of the nuclei, the boundaries are at once lost and nothing but a clear space can be found. The protoplasm peripheral to the cavity is therefore comparatively thin, and does not present the granular structure of the deeper lying regions. The cavities in many cases extend laterally upon either side some distance from the superficial line of contact of the two cells, and sometimes, as in the cell $c^{5.3}$, Figure 28, they even lie between the nucleus and the external surface of the cell. In all cases it is possible to detect a sharp and definite boundary to these cavities, when the egg is so oriented as to bring the margin of the cavity into the proper relation to the optical axis of the microscope. These boundaries have the same appearance in whole preparations and in sections that cell boundaries have, and indeed I believe that they are cell "membranes," and that the cavities are strictly *intercellular.* That part of the facet of contact lying peripherad to the cavity is not continuous through the cavity with the part centrad (Plate III. Figs. 24, 25), but is in direct continuity with the wall of the cavity. This seems to me to be indisputable proof that these vacuoles are intercellular structures, just as the lenticular spaces and central cavity of the earlier stages of cleavage and the large cavity of the twenty-four-cell stage are. The question as to whether these should be called the cleavage cavity will be discussed later.

The appearance of these cavities in section is shown in Figures 24 and 25 (Plate III.). The egg here represented is a very small one, only 80 μ in diameter, and is shown *in toto* in Figure 23. It has just been derived from the sixteen-cell stage by the division of the quartets 5.1 and 5.2. Traces of this division can still be seen in the derived quartets 6.1, 6.2, and 6.3, 6.4. The sections were cut obliquely to the vertical axis, and so directed as to cut longitudinally the remnants of the spindles in one of the quadrants of the quartets 6.3 and 6.4. There is a medium-sized central cavity, which, owing to the recent division and consequent rounded condition of the cells concerned, lies nearer the vegetative pole.

The section shown in Figure 25 cuts the cells of the animal pole obliquely, and thus exaggerates their relative size somewhat. In addition to the central cavity, there are a number of smaller cavities between the cells of the animal pole. Their relation to the cell boundaries can in every case be readily determined in the sections. The larger cavity *x* of Figure 24 is between two cells whose facet of contact lies parallel to the plane of the section ; the cavity therefore appears to traverse a cell, though in reality it does not. In the figure it is in direct continuity with cavities which are readily seen to be intercellular. These latter cavities appear lenticular in cross section, but they are themselves elongated as the cavity *x* is. There are, in addition to the intercellular cavities just described, two others (*vac.*, Figs. 24 and 25), which seem to be *intracellular*). They are both near the central cavity, though not in direct contact with the cell membrane in any direction. They are both approximately *spherical* in form, and neither has the sharp and definite outline separating it from the protoplasm of the cell that the intercellular spaces just described have. Their form, position, and limits thus indicate their intracellular nature. They probably are merely intracellular vacuoles. Their position is suggestive of their fate. They lie very near the central cavity, and it may be that their contents ultimately find their way into it by osmosis, or less probably by rupture of the "cell wall."

The fate of the fluid eliminated from the lenticular spaces of the earlier stages, and from the intercellular spaces of the later stages, is a difficult matter to determine. Direct observation gives negative results, for although these cavities in the early stages can be seen to develop and disappear, it is impossible to say what becomes of their contents. I have never seen any evidence of the extrusion of their contents from the egg, but this might escape observation, for the cavities are small and their contents are transparent, and it is often difficult to determine the point at which the contents even of the large cavity are expelled.

There are, however, some facts which lend support to the view that these lenticular spaces of the early stages, and perhaps also the intercellular spaces of the later stages, contribute directly to the increase of the volume of the central cavity. The nuclear conditions of Figures 23–25 (Plate III.) show beyond question that this twenty-four-cell stage is younger than that of Plate V. Fig. 34, where the larger cleavage cavity is shown. It seems reasonable to derive the conditions of the older stage from those of the younger. The main difference between the two stages lies in the increased size of the cleavage cavity and the absence of the secondary intercellular cavities in the older egg. It seems probable that, as the central cavity grows in volume and the facets of contact diminish in size, the central cavity extends to these secondary cavities and fuses with them, and that thus all portions of the surfaces of the cell, except its exterior one, may contribute secretions to the central cavity. The immediate proximity of several of these secondary cavities to the central cavity in Figures 24 and 25 (Plate III.) suggests that the former may even actually move toward the central cavity. The direction of the motion is merely a question of the direction of least resistance; it is difficult to explain the development of such a large cavity as that of Figure 34 (Plate V.) and the subsequent forcible expulsion of its contents, and the immediate restoration of the egg to a solid spherical mass, without admitting the existence of a considerable force, tending to preserve intact the contour of the egg, and resisting the increasing tension brought about by the enlargement of the central cavity. If the contents of these smaller cavities are eliminated to the exterior, why should not those of the large cavity, whose tension must be proportionally greater, be eliminated at the same time? There is a point, however, beyond which the increase in the size of the central cavity cannot go. The outer wall yields to the pressure, and the imprisoned fluid escapes. I have found no trace of an egg membrane, such as Gegenbaur ('52) has described for the egg of Limax agrestis: "Es besitzt eine Zellmembran, die besonders durch längere Einwirkung von Wasser deutlich erkennbar wird." There is no evidence that there is anything more than the ordinary clear stratum of protoplasm at the exterior of the egg. In this my observations are in accord with those of Dr. Mark upon Limax campestris ('81). None of these secondary cavities or lenticular spaces were observed in the eggs which have the maximum central cavity, neither have they been seen at the time of the expulsion of the contents of the cavity, even when that takes place gradually. They are associated with the growth rather than with the disappearance of the central cavity. In view of these facts, it seems to me that we are justified in concluding that, in some cases at least, the lenticular and the secondary intercellular spaces contribute to the increase of a central cavity.

As has been stated already, these secondary intercellular spaces often form at the animal pole of the egg, while not a trace of them can be found at the vegetative pole. They may present the appearance of an anastomosing network of irregular vessel-like structures between the cells of that pole of the egg, as in Plate VI. Fig. 39. It hardly seems possible that a histological differentiation can have already taken place between the two poles of the egg whereby the cells of the animal pole are set apart to perform an excretory function. This is rendered still more doubtful by the frequent occurrence of eggs in which these secondary intercellular spaces have reached an enormous development at both poles, in fact throughout the whole egg. This condition may occur as early as the twenty-four-cell stage. In such eggs there is never any distinct central cavity present; it becomes difficult in such cases to locate cell boundaries and the relation of nuclei to them. In Plate III. Fig. 26, is shown a transverse section of such an egg containing more than one hundred cells. In stainability and nuclear conditions this is not essentially different from other eggs; several cells of this egg are in a mitotic state; I therefore believe such eggs to be normal. As can be seen in the figure, the three germ layers are present, and the vacuolation surrounds the cells of all three layers indifferently. There is no central cavity, and the three layers retain their connection with one another. Indeed, this condition is very suggestive of that found in the gastrula at the time when the head-vesicle is beginning to develop and the entodermal cells are sending out long processes into the fluid-filled space toward the cells of the other layers. It seems therefore no misuse of terms to designate the intercellular spaces in both cases as the primary body cavity, which throughout the period of segmentation is also the *cleavage cavity*. The condition represented in the figure is ephemeral and the extrusion of the liquid contents may take place without the formation of a spherical central cavity. The spaces seem to be thoroughly connected with one another and when some point on the periphery of the egg yields to the pressure, the fluid is probably in large part eliminated.

The occurrence of a single distinct central cavity is shown in Figure 47, a section of an embryo of eighty cells, and likewise in Figures 48 and 49 (Plate VII.), where the embryo has assumed the flattened shape characteristic of the stage preceding gastrulation. In this egg the cavity is small and lies between the ectoderm, the entoderm, and the bilaterally placed mesoderm bands. There is no trace of any cavity in the mesoderm. In Figure 54 the cavity occupies a position at the posterior end of the blastopore, and, as in the preceding stage, lies next to the ectoderm on the dorsal side of the embryo. I have found many embryos, not figured, which have this definitely limited central cavity. In no case, however, has it attained the size of the cavity in the twenty-four-cell stage shown in Plate V. Fig. 34. On the other hand, a large part of the embryos examined have either the intercellular vacuolation or no trace of any cavity whatever.

What is the morphological and what the physiological significance of the phenomena with which we have been dealing? Are these cavities all simply different phases of one and the same thing, — an ephemeral recurrent cleavage cavity, — or do they differ among themselves, and are they to be considered as different from the cleavage cavity? Do they bear any relation to the conditions under which the egg develops? My work has left no doubt in my own mind that they all belong to the same category, — modifications of the cleavage cavity, — and that they are perhaps intimately connected with the conditions in which the embryo develops. I shall refer to these points again after reviewing the literature of the subject.

The question might well be raised as to whether the term cleavage cavity should be used to designate the phenomena which we have discussed. If we are to apply this term to that continuous, persistent cavity into which the invagination resulting in gastrulation takes place, and that only, then we assign to the term an unduly limited morphological significance, suggested by the Gastræa Theory of Haeckel. Then this ephemeral cavity in Limax is not a cleavage cavity, and we must coin some new term to distinguish it, such, for example, as excretory cavity. If, on the other hand, we recognize the physiological importance of this and other cleavage cavities, while not denying their morphological significance, and bear in mind also the constant intercellular nature of the phenomena in question, it is in my opinion perfectly legitimate to designate by the term cleavage cavity any and all of the protean forms which the intercellular space assumes in Limax. The matter of terms is, however, a minor point, the unity of the phenomena is the important one.

There remains one more question of interest. Is there any relation between the stages of cleavage and the recurrence of the cavity? Warneck, in 1850, stated that the cavity reaches its greatest development contemporarily with the "Maximum der Entwickelung der Kerne." My own observations do not show that this is always the case. In Figure E, the two cells enclosing the large cavity contain, not nuclei with distinct membranes, but spindles. There is a mechanical cause for the elimination of the contents of the cavity at the period when the cells assume a rounded condition at the close of cleavage. The facets of contact are then much reduced, and the resistance at the periphery of the egg to the expulsion of the fluid is more readily overcome. It may also be that the periods of great activity in the cells at the time of division are periods at which the osmotic processes reach a maximum, and thus the cleavage cavity may grow rapidly at this time. My observations on living eggs show that the period immediately preceding division is that of the most rapid growth of the cavity. It is not an uncommon thing to find in the later stages neighboring cells in a mitotic state enclosing a lenticular space between them. These two causes may result in producing in some cases, during the early stages of cleavage, an apparent rhythm between the nuclear conditions and the periods of expulsion. There is, however, much variation in these early stages, and it is impossible to establish in them any such constant correlation as Warneck has indicated.

2. *Literature.*

AMPHINEURA.

No mention is made of any cleavage cavity in the development of Dondersia, as described by Pruvot ('90). Kowalevsky ('83) does not discuss the subject in Chiton, but Metcalf ('93) describes the cleavage cavity as already formed at the four-cell stage. No statement is made about its subsequent disappearance.

LAMELLIBRANCHIATA.

I. *Marine Forms.*

Lovén ('48) does not figure a segmentation cavity in either Modiolaria or Cardium. Barrois ('79) makes no reference to any segmentation cavity in Mytilus, though his Plate XII. Fig. 16, if it represents a *section,* shows such a cavity. He distinctly states that the segmentation produces a body considerably larger than the original ovum. He also notes in the two-cell stage the appearance, in one instance, of lenticular refractive bodies apparently identical with those figured by Bobretsky as found in Nassa mutabilis. These bodies are adjacent to the furrow separating the micromere and macromere of the two-cell stage, and may be due to a highly refractive secretion accumulated in these regions.

Brooks ('80^{a}) describes in Ostrea Virginiana a transparent cavity separating the ectoderm from the macromere *in dead eggs* at a stage when the macromere is almost covered by the very large number of ectoderm cells present. He does not regard this space as normal, since the macromere seems in living eggs to be in contact with the outer layer, and there is no indication of a segmentation cavity. It is only concerning a later stage, when the macromere has divided into a number of entoderm cells, and the embryo is much flattened in the dorso-ventral direction, that he says a distinct "segmentation cavity, or more properly a body cavity, is now clearly visible" between the ectoderm and the entoderm. Horst ('82) says, "It is not possible to demonstrate the existence of a true cleavage cavity in the oyster."

Hatschek ('80) finds that one of the peculiarities of the development of Teredo is "der gänzliche Mangel der Furchungshöhle."

II. *Fresh-Water Forms.*

Forel ('68, p. 14) called attention to the fact that the "yolk" in Unio later becomes clear and transparent, but he failed to interpret this appearance as a cavity. It remained for Flemming ('75) to show in Anodonta the presence of a cleavage cavity as early as the two-cell stage, to establish its recurrent character, and to assert its equivalency to the cleavage cavity of the later stages. He notes its formation as a lens-shaped cavity between the macromere and micromere of the two-cell stage; its disappearance with approaching cleavage; its reappearance in the four-cell stage, and its subsequent disappearance before the next cleavage begins. He also notes its relatively large volume in a stage when there are from six to ten micromeres, but he does not describe any further obliterations of the cavity. The fact that the earlier cavities are obliterated does not raise the question with him as to whether they should be regarded as cleavage cavities or not.

Rabl ('76) has observed a similar phenomenon in Unio pictorum. He describes the cavity as a transparent protoplasmic layer with few yolk granules between the cells of the two-cell stage. He contends, however, that it is not the beginning of the cleavage cavity, as Flemming had maintained, and calls attention to the fact that similar regions, free from granular structures, between two or more cleavage spheres, are met with in the embryos of other animals, as, for example, in many Gasteropods, where the cleavage cavity appears later than it does in Unio. At the four-cell stage, however, he recognizes "die erste Anlage der Furchungshöhle," but does not speak of any reduction or disappearance of this cavity in later stages, though some of his figures suggest it.

Korschelt ('91) speaks of an expansion of the primitively narrow segmentation cavity of Dreissena, during which the embryo, which has reached the gastrula stage, assumes a roundish oval shape. No mention is made of the ephemeral or recurrent character of this primitive cavity. It is a matter of considerable interest to see that Dreissena, which is a "near ally of the common mussel," and is probably a recent migrant into a fresh-water environment, still retains the free-swimming larval stage characteristic of marine forms. It has acquired, however, the "primitive segmentation cavity" found in the fresh-water Lamellibranchs, but not definitely known to be present in the marine forms.

Lankester ('74) does not refer to the cleavage cavity of Pisidium, nor does he figure it except in comparatively late stages of development.

Von Jhering ('76) speaks of the three or four small cells in Cyclas, whose progeny grow around the solid mass of the two large cells, and of the later appearance of a cavity in the centre of this mass. Ziegler ('85) finds a cavity in the thirteen-cell stage of Cyclas, but indicates no cavity in the two earlier stages that he figures.

The latest, and by far the most important, contribution to our knowledge of the cleavage cavity is that of Stauffacher ('93) upon Cyclas cornea. The formation of a "true" cleavage cavity takes place at the thirteen-cell stage by the gradual elevation of the cap of ectoderm cells from the macromere to which they had been closely applied, resulting in the development of a sharply defined space between the macromere and its derivatives. This cavity persists and increases in size until it ultimately becomes the relatively very large cavity of the blastula stage. In addition to this cavity, which he regards as persistent from the thirteen-cell stage on, Stauffacher finds in the two-cell stage a structure which he regards as similar to that observed by Flemming in Anodonta and by Rabl in Unio. He describes it as a disproportionately large space, entirely unstainable, *in* the smaller of the two spheres, exactly in the region where they are in contact. The cavity is filled with a fluid free from granules. The protoplasmic part of the cell, which forms the peripheral layer and contains the nucleus, merges very gradually into this fluid-filled space. On the side of the macromere this space is sharply and definitely limited. It seems from his description that this space is regarded by him as lying *in* the smaller cell, i. e. intracellular, though he does not distinctly designate it as such. The interpretation of this space and its later history are best given in his own words (*loc. cit.*, p. 211): "Es fällt bei Cyclas nicht schwer, den unumstosslichen Beweis zu erbringen dass der genannte helle Raum in der That nichts mit einer Furchungshöhle zu thun hat. Dieselbe körnerlose Partie nämlich, die wir auf dem zweizelligen Stadium antreffen, ist zwar auch auf dem dreizelligen Stadium noch vorhanden, aber schon bei der Bildung der vierten Furchungskugel wird sie bedeutend reduziert und verschwindet schliesslich ganz. Dagegen entstehen im weiteren Verlauf der Entwickelung zwischen der grossen Mutterzelle und ihren jeweiligen letzten Abstammungsprodukten neue solche Partien. Die eigentliche Furchungshöhle tritt bei Cyclas, wie wir sehen werden, erst in bedeutend höheren Furchungsstadien auf."

A cavity similar to that of the two-cell stage is figured for the four-, five-, six-, seven-, nine-, and twelve-cell stages, occurring always between the macromere and its most recent products. This cavity becomes successively smaller from the four- or five-cell stage until we reach the relatively small cavity of the twelve-cell stage. It is always sharply limited from the macromere, and often presents on the side next the most recent micromere, or its products, the gradual merging into the granular protoplasm noted in the two-cell stage. That is to say, here, as there, the inference is that the cavity may be regarded as an intracellular space. The fluid which fills this decreasing cavity he thinks is absorbed in large part by the macromere, and perhaps to a less extent by the micromeres, and that it does not pass out of the cavity through the egg membrane. After this fluid-filled space has disappeared from between the earlier formed micromeres, m^1, m^2, m^3, m^4, and the macromere, the micromeres in question apply themselves closely to the macromere in a way that suggests the fusion of micromeres with the macromere noted, as by Lovén ('49) in marine Lamellibranchs, and by Bobretsky ('77) in Nassa. With regard to the interesting phenomenon of fusion described by these authors, Stauffacher makes the following suggestion: "Es erscheint mir nicht unwahrscheinlich dass vielleicht in allen den Fällen, wo ein nachträgliches Abflachen der kleineren Zellen konstatiert wurde, auf günstigen Prepäraten auch der helle Raum zwischen den Furchungskugeln hätte nachgewiesen worden können, der durch sein Verschwindung das Anschmiegen der Mikromeren möglicherweise bedingt." "Der helle Raum" has, however, never been recorded by any investigator of these forms; furthermore, the fusion in some cases (and these are the most marked cases of fusion) consists in the reunion of the more richly protoplasmic part of the macromere with the more passive yolk-bearing portion, from which it had abstricted itself at the time of nuclear division.

Neither Bobretsky ('77) nor Brooks ('80) figures a nucleus in the "macromere" with which the micromere so completely fuses; and it seems hardly possible that in these cases the disappearance of a cavity can have anything at all to do with the phenomenon of fusion. There are moreover some objections to the view that in the two-cell stage the cavity lies *within* the cell, and to the inference that it is essentially of that nature in the later stages. Stauffacher himself does not emphasize, or even clearly present this view, though he repeatedly calls attention to the lack of a sharp differentiation of the cavity from the protoplasm of the most recent derivative or derivatives of the macromere. The fact that this gradual transition is shown toward *two* cells, as in his Taf. XII. Figg. 14 *a–g*, and Taf. XIII. Fig. 18 *b*, militates against the view that the cavity is intracellular. It may well be that the yolk-laden macromere, on account of its different stainability, is more sharply marked off from the cavity than the protoplasmic micromere; but is it not possible that the gradual transition of the granular protoplasm of the adjoining cell into the clear space of the cavity is in most, if not all, of the cases figured by Stauffacher due to oblique sections of the limiting membrane? His figures of the whole egg are made from reconstructions on glass plates, and in them the outlines of the cavities are not distinctly traced. In most cases he has not indicated the planes of the sections which he figures; these must therefore be inferred from the position of the nuclei. Such inferences, however, lead one irresistibly to the conclusion that the sections must meet the boundary of the cavity obliquely wherever its outline appears indistinct; e. g. Taf. XII. Figg. 14 *a–g;* Taf. XIII. Figg. 18 *a* and *b*. On the other hand, sections which appear to strike the cavity perpendicularly, as in Taf. XII. Figg. 15 *a* and *b*, 16 *a* and *b*, and 17 *a*, all show a much more distinctly marked separation of the protoplasm of the cells from the cavity, and in some cases this demarcation is as definite on the side of the most recent micromere as it is upon that of the macromere. In case this explanation should prove valid, we shall have in Cyclas, as in Limax, an intercellular cavity appearing at the two-cell stage, and recurring in the later stages of cleavage.

I cannot agree with Stauffacher's view that this primitive "heller Raum" has nothing whatever to do with the true cleavage cavity. It is not established even by the facts found by him in Cyclas; much less by a comparison with other forms presenting a similar phenomenon. His observations are confined to killed, preserved, and hardened material of very limited amount. He had in some cases not more than one series of sections of each cleavage stage; of the three-cell stage seven series, of the four-cell stage six series. He has not been able to examine the eggs in the living state, or in whole preparations. Thus he has been deprived of most valuable assistance in determining the origin, definite boundaries, successive phases, ultimate fate, and relationships of this "heller Raum," whose claim to the title of cleavage cavity he so summarily dismisses. The "unumstosslich Beweis" which he brings forward to support the view he advances is, that the "heller Raum" finally disappears entirely. But his figures and descriptions show that this clear area merely occupies a different position with reference to the first micromeres, not that it entirely disappears. Every stage that he figures, from the two- to the thirteen-cell stage, where, upon his interpretation, the true cleavage cavity first appears, contains a cavity. He brings forward no proof to show that these may not be continuous both with one another and with the cavity of the thirteen-cell stage, which is in his view the true cleavage cavity. It seems to me, then, that his own evidence does not conclusively sustain the view that this primitive cavity is not a true cleavage cavity, as he himself has defined it. Indeed, we should expect that in Cyclas, as in other fresh-water mollusks, there might be an entire elimination of the cavity at intervals, though he has not proved it. Even if we grant that in Cyclas the primitive cavity is eliminated, we have still the important point to consider whether or not such an elimination constitutes a valid ground for removing the "heller Raum" from the category of cleavage cavities. A comparison of the phenomena in Cyclas with those presented in such a form as Limax would seem to indicate that we are dealing here, as there, with an ephemeral recurrent cleavage cavity present at the very beginning of segmentation.

Scaphopoda.

Kowalevsky ('83) finds a definite cavity appearing in Dentalium as early as the eight-cell stage. This gradually increases in size, forming quite a large cavity at the time of gastrulation.

Prosobranchiata.

I. *Marine Forms.*

An examination of the literature of Prosobranch development shows an almost entire absence of references to a cleavage cavity. The few allusions that exist are concerned with the cavity that appears comparatively late in the period of cleavage.

Bobretsky ('77) finds a cleavage cavity in Nassa mutabilis at the thirty-six-cell stage. Although the alternation of the rounded with the flattened conditions of the cells in cleavage is quite prominent in Nassa, no cavity is noted as occurring between the fused cleavage spheres.

McMurrich ('86, p. 412) makes the following statement with regard to the segmentation cavity in Fulgur: "To one side of the blastoderm and below it a more or less distinct cavity is to be seen, containing granular matter. It is possible that this may represent the segmentation cavity, though it does not appear to be present in all cases." This is in a stage preceding the formation of the "sixth generation" of micromeres and the appearance of the mesoderm. Brooks ('78) figures at a late stage a cavity in Urosalpinx similar to that found by Bobretsky in a late stage of Nassa. Patten ('86) does not figure any segmentation cavity in Patella, but in the later stages, before gastrulation, a medium-sized central cavity is present. Conklin ('91) finds in Crepidula, "at an early period, a trace of a segmentation cavity, which, however, is soon obliterated."

II. *Fresh-Water Forms.*

Blochmann ('81) does not discuss the subject of the cleavage cavity in Neritina, but it is evident from his figures that, if it is present at all in the earlier stages, it does not attain a great size. Neritina contains a large amount of yolk, and this may have some effect on the cleavage cavity. In the late stages a small cavity appears between the ectoderm and the macromeres.

No mention is made by Sarasin ('82) of a cleavage cavity in Bithynia until the close of the cleavage period. Sections of the early stages were not cut. Erlanger ('92) finds a large cavity present at the close of segmentation, i. e. before the formation of the mesoderm and when the blastomere contains, according to his estimate, at least sixty cells. In Paludina vivipara, Lankester ('76) finds in a late stage of cleavage "a central space or cleavage cavity." A cavity of considerable size is also figured by him as present at the time of gastrulation. On the other hand, neither Bütschli ('77) nor Blochmann ('83) succeeded in finding in this species any cavity in the cleavage stages examined by them, nor more than a mere slit-like cavity between the layers at the time of gastrulation. Erlanger ('91), however, finds a large cavity in the gastrula stage, and it is into this cavity that the mesodermal pockets described by him are evaginated. If Paludina has an ephemeral recurrent cavity similar to that of Limax, the apparently discordant observations of Lankester, Bütschli, and Blochmann would be easily harmonized.

I have myself watched the cleavage of the eggs of Amnicola limosa, and find that they present a typical recurrent cavity, precisely like that of the fresh-water pulmonates. The eggs of Amnicola are enclosed in capsules and are surrounded by a jelly-like albumen. They contain a small amount of yolk, and cleavage is not so unequal as it is in Neritina.

OPISTHOBRANCHIATA.

There seems to be an entire absence of references to the presence of a cleavage cavity in the development of this group.

Heymons ('93) found in Umbrella no trace whatever of a cavity at any period up to the formation of the larval stage.

PTEROPODA.

Fol ('75) says of the two-cell stage of the Pteropods, "Mais il ne se produit pas ici, comme chez le Lymnée et la Limace, ou comme chez les Geryonides, des vacuoles entre les cellules." Nor is a cleavage cavity described by him for the later stages. Knipowitsch ('91) mentions a "spaltförmige und nicht immer deutlich wahrnehmbare Furchungshöhle," into which the mesodermal cells migrate, as occurring at the end of cleavage in Clione.

PULMONATA.

Warneck ('50, pp. 131–135, 166–170) discusses the recurrent cleavage cavity in Limax and Lymnæus. He describes its appearance soon after the two cells begin to flatten against each other; also its growth and subsequent disappearance when the second cleavage plane appears. A similar phenomenon occurs at each succeeding phase of cleavage till the blastula stage is reached. He expresses the opinion that this "heller Raum," as he calls it, is a receptacle for albumen, and describes the expulsion of its albumen-like contents into the surrounding albumen at the time of the disappearance of the cavity. He explains the phenomenon as due to the acceleration of end- and ex-osmosis, attendant upon the greater activity of the nutritive and excretory functions of the cells and the disappearance and reappearance of the nucleus during the successive phases of cleavage, and correlates this activity of the cells with the origin of the ovum from a glandular tissue. Ganin ('73) mentions the relatively small cavity in Lymnæus, and the larger cavity in Physa. In Helix, von Jhering ('75) finds a central cavity in the two-cell stage. The later stages of cleavage were not carefully followed by him, and no further mention of the cavity occurs in his work.

Fol ('80, pp. 115 and 116) says: "Pendant le travail du fractionnement, les sphérules prennent un aspect foncé et une forme arrondie. Les noyaux ne sont plus visibles et la cavité de fractionnement se perd dans l'obscurcissement de l'œuf. Pendant les temps de repos les noyaux reparaissent, les sphérules s'affaissent les unes sur les autres, la cavité de segmentation est de nouveau visible. Dans ces périodes de repos, la limite entre les sphérules apparaît comme une ligne d'épaisseur très appréciable et dont la transparence contraste avec la teinte foncée des cellules ; c'est la coupe optique de la couche de sarcode qui règne sur toute la surface des sphérules. Mais en outre nous remarquons dans le plan de contact des cellules voisines une accumulation de liquide, fait déjà constaté par Warneck. Ce liquide va sans doute remplir la cavité de fractionnement, mais une partie est aussi expulsée sous forme de gouttelettes qui sortent généralment au pôle nutritif. J'ai observé une seule goutte chez les Hétéropodes et les Pulmonés aquatiques. . . . La cavité de segmentation, ou blastocèle, reste presque nulle pendant que l'invagination se forme et ne commence à croître qu'après le rétrécissement du blastopore."

The propriety of the use of the term blastocœl or segmentation cavity to designate the lacunar spaces of the mesenchyma of the gastrula, as well as the spaces between the primitive blastomeres, is questionable. To be sure the mesenchymatous lacunæ are derived from the blastocœl rather than from an enterocœl, in Limax at least ; but our choice of terms is not limited to blastocœl and enterocœl, and it seems preferable to apply to those spaces without epithelial lining which lie between the ectoderm and entoderm, and are traversed by loose mesenchymatous cells and prolongations of the ectoderm cells, a term not already set apart for another use. Previous to the formation of these lacunæ, all the cells of the intermediate layer exist as a solid mass obliterating the cleavage cavity. When, however, in Limax the cavity reappears, as in Figure 54, it is as a distinct space bounded by germ layers. It seems better, therefore, to apply to the spaces mentioned in the second quotation from Fol (p. 116) the same term which is used elsewhere for mesenchymatous lacunæ not lined by a distinct epithelial layer, viz. schizocœl.

Rabl ('79, p. 568) notes the presence of a cleavage cavity in the twelve-cell stage of Planorbis, and suggests the possibility of its presence in the eight-cell stage. It attains its maximum size when the embryo consists of twenty-four cells. He mentions the flattening of the blastosphere which follows this maximum condition, but does not speak of an obliteration of the cleavage cavity accompanying it, and considers that the cavity is still present at the time the mesoderm cells sink below the surface. He makes no suggestion of its recurrent nature at any period of its existence.

Brooks ('80, p. 80) mentions in Physa the " presence of a lens-shaped segmentation cavity, which is enclosed peripherally by the union of the two primary segments. This cavity persists from this stage until the completion of segmentation." He does not refer to its recurrent character in the earlier stages of cleavage, nor to the elimination of its contents in whole or in part.

Joyeux-Laffuie ('82), in his work upon Oncidium, a marine form with pulmonate affinities, makes no reference to a cleavage cavity.

From my own observations on Planorbis and Physa, I have no doubt that the recurrent segmentation cavity is found in these forms, as in Limax; but it is not developed in so marked a degree. I wish in this connection to call attention to the fact that the enclosing capsules and albumen of these forms are less dense than those of Limax, and that they are deposited *in the water.* In Planorbis, which has somewhat more yolk than Physa, the cavity does not attain so great a size as in Physa.

I shall not enter into an extended discussion, or a review of the literature of the cleavage cavity in other groups of animals, especially of marine forms. I shall refer mainly to those forms which, by reason of their conditions of development, might be expected to throw light on the significance of the cleavage cavity.

In Spongilla, likewise a fresh water animal, Maas ('90) finds no trace of a cavity in the solid morula stage, though he admits that there is at the four-cell stage the intimation of one, which later entirely disappears.

According to Brauer ('92) a cleavage cavity appears in Hydra at the eight-cell stage, but he makes no reference to a subsequent disappearance of the cavity.

ROTIFERA.

Zacharias ('85) finds a cleavage cavity in the two-cell stage of Philodina roseola. He does not figure it in the later stages, but speaks of its general appearance in all the eggs whose early stages he had observed.

Zelinka ('91) does not figure any cleavage cavity in the development of Melicerta or Callidina.

ANNULATA.

I have found no reference to a recurrent cavity in the marine forms of this group. In forms with much yolk, as Nereis, there may be no cavity whatever (Wilson '93); but in forms whose division is nearly equal, as in Eupomatus, a cavity appears at an early stage and persists until gastrulation (Hatschek '86).

Whitman ('78) describes a cavity in Clepsine, which appears very early at the place where the first three planes of division cross one another. Its early and later history is not given. No mention is made of any obliteration or recurrence of the cavity. He suggests that "the blastocœl, whenever it appears, forms as a necessary result of the cleavage process. . . . The explanation of the cleavage cavity depends upon the fact that the cells push each other apart in cleaving."

This explanation was doubtless suggested by the cleavage of Clepsine, where there is considerable difference in size between the micromeres and macromeres, and the less mobile yolk-laden cells adapt themselves less readily to the changes in cleavage than the protoplasmic blastomeres of the egg of Limax. The difficulty of applying this mechanical explanation of the cleavage cavity to the phenomena observed in Limax will be patent to all. The blastomeres, in this form at least, are exceedingly plastic bodies, adapting themselves either to the presence or absence of a cavity, upon which profound changes in their form depend. Furthermore, the "pushing apart" of the cells in cleavage is often the occasion of the obliteration of a cavity rather than its formation; for the cavity, in the early stages at least, is frequently at its maximum just before cleavage, and is obliterated or reduced in size at its close.

Wilson ('89) finds in the four-cell stage of Lumbricus a cavity which he labels "segmentation cavity," and of which he says, it "disappears afterwards and cannot be identified with the true blastocœl," which is described for the thirteen-cell and later stages. The eggs of L. fœtidus, for which this ephemeral cavity is figured, have tough capsules and thick albumen, similar to that of Limax.

Vejdovsky ('88–'92) describes a distinct cavity in the two-cell stage of Rhynchelmis, and refers to the occurrence of a similar cavity in later cleavage stages. It is evident, however, from his figures, that the cavity is not so prevalent as it is in Limax. A similar cavity occurs in the two-cell stage of Allolobophora. A very interesting phenomenon was also observed by him in the six-cell stage of Allurus tetraëder. In the cytoplasm of the micromeres of this stage, a number of large contorted canals appear, which resemble very much the canals found later in the larval excretory cells of the embryo. These canals are filled with a clear fluid and "hängen offenbar zusammen." The accumulation of fluid in the canals results in an increase in the volume of the micromeres, and an *obscuring of* both *cell boundaries* and *nuclei*. Finally, by a powerful contraction the fluid contents are expelled and the micromeres assume their original form and size, the cell boundaries and nuclei again becoming distinct. It is evident that the author regards these canals as intracellular structures. No sections of this stage are figured, and the relation of these canals to cell boundaries is not determined. The fact that the presence of the canals obscures the boundaries between the cells, and that these canals are in continuity, suggests the possibility that they may be *inter*cellular and therefore merely an exaggerated form of the anastomosing intercellular spaces so common in Limax. Vejdovsky does not suggest their relationship to the cleavage cavity, neither indeed does he regard a space found in the two-cell stage as having anything to do with that cavity. His grounds for this view, and his explanation of the phenomenon, are as follows (p. 105): "Die Höhle zwischen beiden Furchungskugeln ist als Ueberrest der Vorgänge zu betrachten, die sich bei der Bildung der Zellmembranen beider Furchungskugeln abgespielt haben. Diese Höhle zwischen den ersten 2 Furchungskugeln ist bereits oft beobachtet und als eine primäre Furchungshöhle (!) gedeutet worden. Es ist überflüssig eine solche Auffassung zurückzuweisen, einmal, dass es unmöglich ist, dass eine Furchungshöhle bereits zwischen zwei ganz gleich gestalteten Furchungskugeln zum Vorschein kommen könnte, ein anderesmal, dass derartige Höhle öfters auch während des späteren Furchungsprocesses zwischen je zwei Kugeln zum Vorschein kommt (vergl. Taf. IX. Fig. 11, 14). Gewiss ist diese Erscheinung von den Verhältnissen der Zell- und Kern-platte abhängig."

In the absence of the evidence upon which these opinions rest, it seems superfluous to discuss them. His suggestion that the formation of the cavity of the two-cell stage is dependent upon the phenomena of the division resulting in that stage is certainly not sustained by the facts. If his opinion were the correct one, we should find a similar cavity in the two-cell stages of all forms, fresh-water and marine alike.

The preceding review of the literature shows that Warneck ('50), Rabl ('79), Fol ('80), and Brooks ('80) have all noted the recurrent character of a cavity in the *early* stages of cleavage in the Pulmonates, but the three later writers have added little to the admirable observations of the first named investigator.

A glance at the summary of the literature on Prosobranch development shows an entire absence of any reference to a recurrent segmentation cavity in the marine forms, unless an exception be made with regard to McMurrich's observations on the cavity in Fulgur. When a cleavage cavity does occur, it appears at a very late stage in the segmentation, is comparatively small, and is never recurrent. The cleavage of the fresh-water Prosobranchs has not been fully studied except in

Neritina, but the cleavage cavity does not seem to be prominent here. It is however well marked in Amnicola.

Among the Lamellibranchs there is the same absence of reference to a cleavage cavity in marine forms, but its recurrent nature is noted in Unio and Anodonta, probably also in Cyclas. A cavity also occurs in Dreissena, but we do not know that it is recurrent. It seems probable that the encapsuled eggs of the non-marine Chætopods may also present cases of a recurrent cavity.

So far, then, as the literature and my own observations go, it seems that this recurrent cavity is confined to eggs developing in *fresh water or moist situations,* and reaches its maximum manifestation in forms enclosed in thick encapsuled albumen, like Limax. In these outward conditions of environment probably lies the explanation of this phenomenon. It is probably correlated with the nutritive and excretory processes of the egg, especially the excretory, as Warneck long ago suggested, for we have no evidence that the embryo depends in the cleavage stages upon the surrounding albumen for its nutriment. The latter serves mainly as a protective covering during these stages, though later it is certainly used as food by the growing embryo. The metabolic processes taking place in the protoplasm of the blastomeres may be attended by the endosmosis of water from the surrounding albumen and its subsequent exosmosis. That part which passes out from the cell along the facets of contact with the other cells or on its inner surface, when such exists, contributes toward the formation of a cleavage cavity in some of its varying manifestations. When however the egg, or its envelopes, is bathed by a changing medium, as is the case with fresh-water and marine forms, the cavity is reduced or is altogether wanting, it being probable that the changing medium facilitates the solution and removal of the waste products from the surface of the egg. This is especially true of marine forms where the presence of the salt in the bathing medium doubtless facilitates the solution of the albuminous matters, and it is in these forms that the cavity is never recurrent and rarely present during cleavage. The eggs of the land Pulmonates, lacking as they do the salt, or even a changing medium, have the further disadvantage of a coating of dense albumen. This and its thick envelopes must necessarily impede the processes of excretion and respiration, a hindrance which may be in part removed by the increase of the osmotic surface attendant upon the formation of the cleavage cavity, and also by the forcible removal of the products of excretion by the expulsion of the contents of the cavity. That there exists a physiological necessity for some such process is evidenced by the enormous development of larval excretory and respiratory organs in these encapsuled embryos; for it is in these forms that the primitive kidneys reach a maximum development, and the pulsating head vesicle and pedal sinus (podocyst or amnion of Jourdain) are found.

A large amount of yolk in forms subjected to a fresh-water environment, as Neritina, may conduce to the suppression of this ephemeral recurrent cavity. Its action may be purely mechanical, or it may perhaps be physiological, in that the process of cleavage, and therefore that of metabolism, take place less rapidly in such forms than in cases where there is less yolk and approximately equal cleavage prevails.

In conclusion, then, it may be said that the existence of a cleavage cavity is dependent more upon the physiological necessities of the egg than upon the internal processes of cell division, or the mechanical necessities of cleavage, and that its significance is preeminently physiological rather than morphological.

3. *Experimental.*

Fortunately the effect of salt in the surrounding medium is a matter that can be tested by actual experiment. As the eggs of Limax must be placed for examination in water, — an unnatural environment, — it has seemed best not to employ these, inasmuch as the water itself introduces a disturbing factor. Water very readily penetrates the membranes of the egg of Limax, and the albumen absorbs it so that the eggs become quite turgid. Accordingly, recourse was had to the eggs of fresh-water forms where it is not necessary to remove the eggs from their natural environment for experimentation. For this purpose the eggs of Amnicola limosa and Physa heterostropha were used. An egg mass of Physa showing the early stages of cleavage was divided into two equal parts, one of which was kept in the normal water of the aquarium while the other was placed in a salt solution made from the same water. The eggs were then kept under continuous observation, the phases of cleavage, and the size of the cleavage cavity in units of the ocular micrometer were recorded for both lots of eggs. There is some variation in individual eggs as to the rate of development, so that the observation of a number of eggs is desirable, in order that the predominant condition may be taken as the typical one.

The eggs of Physa in the four-cell stage with a maximum cavity were placed in 0.75% salt solution. The elimination of the contents of the cavity occurred very soon afterwards, and slightly earlier in the eggs in the salt solution than in those under normal conditions. These later proceeded to cleave normally, forming a new cavity after the cleavage, which followed close on the obliteration of the cavity. The eggs in the salt water did not divide, nor did a central cavity reappear, small lenticular spaces only being formed. All further activity seemed to be suspended during the four hours that the eggs were kept under observation. A portion of this lot was removed to fresh water after an exposure to the salt solution for an hour and forty minutes. In the course of an hour more cleavage took place, the cells passed through the rounded and flattened conditions, but during the two hours in which the eggs were observed *no* cavity appeared. These eggs seemed to be in a normal condition on the following morning. Cleavage had not advanced in the least in the eggs which remained in the 0.75% solution over night.

The result of this experiment seems to show that the sudden transfer of the eggs to the 0.75% salt solution is inimical to their further development, and that it may have some influence in retarding the appearance of the cleavage cavity when they are restored to fresh water.

A trial of 0.38% salt solution gave approximately the same results.

When, however, the eggs of both Physa and Amnicola were placed in a 0.19% or 0.10% solution, cleavage was at first slightly delayed, occurring from five to fifteen minutes later than in the control eggs in the fresh water. After this initial delay, the cleavage seemed to progress in both lots with equal rapidity. With regard to the cleavage cavity, however, the two lots of eggs presented decidedly different results. When the transfer was made to the salt solution a maximum cavity was already present in both lots. Elimination occurred approximately at the same time in both, but the subsequent reappearance of the cavity in the eggs in the salt solution was delayed from fifteen to thirty minutes, and it never reached the size of that in the eggs in the fresh water. For example, the cavity in the control eggs attained a diameter of five to seven units, while that of the eggs in the salt solution was only three to four at the time of elimination. There were, however, a very few cases in which the cavity reached a diameter of five units.

These somewhat limited experiments seem to accord very well with the observations on the cleavage cavity in fresh-water and marine forms, and with the interpretation given to the cleavage cavity in the preceding pages. They are also interesting in the light of Gruber's ('89) experiments upon fresh- and salt-water Protozoa. When a species common to both environments, as Actinophrys sol, is transferred from salt to fresh water, there is a marked increase in the vacuolation of the protoplasm.

This same vacuolation is characteristic of the fresh-water forms, as contrasted with forms having a marine habitat. The reverse experiment has an opposite result, — a reduction of the vacuolation in the individual when gradually transferred to salt water. Strictly marine forms, as Amœba crystalligera, also show a marked development of vacuolation when they are brought into fresh water. These phenomena seem to show an increased activity of the protoplasm in the absorption of water, and a corresponding increase in the excretory function is also indicated by the much greater activity of the contractile vacuole in fresh-water as compared with marine forms. The physiological action of the recurrent cleavage cavity of the Pulmonates is strikingly suggestive of the contractile vacuole of the Protozoa. The morphological distinction, that the one is intracellular while the other is intercellular, militates however against the homology of the cleavage cavity and contractile vacuole.

VI. BLASTOPORE AND GASTRULATION.

I have already called attention in the preceding pages to the changes in form characteristic of the stages of cleavage there discussed. The same causes produce similar changes in the later stages. We encounter rounded embryos with a central cavity (Plate VII. Fig. 47), and also much flattened individuals (Plate VII. Fig. 50). The latter are more common, and very generally present a more or less quadrangular outline, the sides of which are parallel to the first two planes of cleavage, i. e. to the antero-posterior and transverse axes of the embryo ; the two or four mesoderm cells are symmetrically placed adjacent to the posterior side.

In the case of two embryos, not figured, the mesoderm occupies a different position with reference to these four sides. Instead of lying adjacent to one of the sides, it lies in one angle, a position suggestive of the condition found in the forty-four-cell stage of Figures 39 and 40 (Plate VI.), where the primary mesoblast does not seem to have undergone a shifting into the median plane of the egg. In these cases it would seem to be necessary to orient the egg after the manner of Blochmann ('81) and Rabl ('79), with the first two cleavage planes cutting the axis of bilateral symmetry at an angle of 45°.

It is in these flattened quadrangular embryos, containing from one hundred to one hundred and twenty cells, six or eight of which are mesoderm, that the first traces of gastrulation occur. Previous to this epoch the ventral face, i. e. vegetative pole, of the embryo has a much more rounded contour than the animal pole. The blastoporic invagination now appears as a broad shallow depression, involving a large part of the median surface of the vegetative pole. It is deeper at its rounded anterior margin and gradually diminishes in depth posteriorly. Figures 43 and 44 (Plate VII.) give the appearance of the depression as seen from the surface at this stage. Figure 48 (Plate VII.) is a sagittal section through the lateral margin of the depression, and Figure 49 is a median sagittal section, the anterior end being at the left in both figures. Figures 45 and 46 (Plate VII.) represent respectively the posterior and anterior regions of the blastoporic tract in transverse section.

The dorsal surface also frequently shows a slight depression at this period. This may be due to the temporary obliteration or reduction at this time of the cleavage cavity, which is bounded laterally by the two bands of mesoderm (Plate VIII. Figs. 46, 48–50). The posterior end of the embryo is now slightly broader than the anterior, Figure 44 (Plate VIII.). The broad ventral depression continues to deepen at the anterior end, and becomes narrower throughout its whole length. This results in a flattened embryo with an elongated median depression deepest at its anterior end.

Such flattened embryos with an elongated blastopore have been figured by Lereboullet ('62) and Lankester ('74) for Lymnæus, and by Rabl ('79) for Planorbis. Fol ('80), who does not figure this stage of the Pulmonate embryo, states that he has not found embryos so much flattened as those described by Lankester. The earlier writers upon Limax have passed over this stage in silence, though Lankester ('75, Plate IX. Figs. 21, 22) figures two gastrulæ of Limax agrestis, both of which appear to be of a later stage, showing considerable difference in size between the cells of the two outer layers.

The anterior border of this deepening blastoporic trough becomes more abrupt, and the lateral borders more sharply marked out, while the depression of the posterior region is gradually obliterated. The most marked change that accompanies this growth of the invagination is the increase in size of the anterior end of the embryo. Viewed from the ventral (Fig. 44, Plate VII.) or dorsal surface, it has had, up to this time, a rounded quadrilateral outline, usually with the posterior end the larger; but in the stages represented in Figures 51–53 (Plate VIII.), the anterior end shows a marked increase in thickness as well as a lateral expansion. The thickening and lateral expansion of the anterior end are brought about by two influences, — the deepening invagination, and the accumulation of mesoderm in the antero-lateral region of the embryo. The blastoporic trough has now closed gradually from behind forward until there is a laterally compressed, cone-shaped pit directed antero-dorsally, and occupying a region at the vegetative pole directly opposite that where the polar globules are located, i. e. the blastopore at this period occupies a position corresponding to the centre of the vegetative half of the egg.

The "landmarks" which assist in the orientation of this stage are: (1) The two lateral masses of mesoderm, now in continuity at the thickened region anterior to the blastopore, but separated posteriorly in the mid-dorsal region, where the ectoderm and entoderm retain their primitive contact. (2) The posterior region of the mesodermal bands, which marks the level of the posterior margin of the blastopore. These lie at the smaller end of the embryo. (3) The lateral projections, which may well be called "velar" projections, for they occupy a position similar to that of the velum in Planorbis. The cells of this region are also somewhat larger than those of the surrounding ectoderm (Plate VIII. Fig. 57). Owing to their constancy and prominence they are a valuable aid in the orientation of the embryo.

At a stage succeeding this, represented in sagittal and transverse section respectively in Figures 56, 57 (Plate VIII.), a considerable transformation has been effected. The whole embryo has increased somewhat in size, owing to the growing vacuolation of the entoderm. The velar projections still persist and serve to emphasize the difference between the anterior and posterior ends of the embryo, as before; but the remnant of the blastopore no longer occupies a median ventral position; it is found at the posterior end of the embryo, Figure 55 (Plate VIII.). The relation of this to the preceding stage is a matter of considerable importance, owing to its bearing on the question of the fate of the blastopore.

It is with considerable hesitancy that the consideration of this perplexing question — the fate of the blastopore — is undertaken, for I cannot bring forward to support my conclusions the decisive evidence that can be offered in other forms, e. g. Umbrella. There are in Limax no definitely fixed and prominent landmarks, such for example as the anal cells of Umbrella. The germ layers themselves are not sharply differentiated from one another by the size of the cells, and the whole matter is further complicated by the existence of the recurrent cleavage cavity, the beginning of the vacuolation of the entoderm, and the development of the head vesicle, all of which contribute to profound changes in the external form of the embryo.

Such evidence as there is seems to lead to the conclusion, that the orifice at this later stage, though posterior in position, is derived from the mid-ventral opening — the anterior end of the blastopore of an earlier stage — by a backward overgrowth of the anterior and lateral margins of that opening, the posterior lip of the blastopore being regarded as fixed, so that the remnant of the blastopore comes to occupy a position corresponding to the posterior lip of the blastopore of the earlier of these two epochs. If this is the case, we should expect to find intermediate stages showing steps in this overgrowth. The earlier stage, Figure 51 (Plate VIII.), shows some trace of it, for the pit of invagination has an antero-dorsal direction, i. e. in a ventral view the anterior lip of the blastopore somewhat overhangs the deeper portion of the invaginated layer, whereas the posterior margin rises obliquely to the level of the ectoderm. This overgrowth is accompanied by an accumulation of mesoderm in the anterior region. Although I have examined hundreds of embryos of about this stage, many of them killed especially for the determination of this question, very few good illustrations of this overgrowth have been observed. Figure 53 (Plate VIII.) is a postero-ventral view of such a stage, showing the thickened anterior end with its velar projections. Occupying the mid-ventral region is an elevation which overhangs the site of the mid-ventral blastopore, whose posterior region is now marked by a trough-like depression. Figure 54 (Plate VIII.) is a nearly sagittal section, slightly oblique, through another egg of such a stage, showing the overhanging anterior lip and its contained mesoderm cells. Owing to the obliquity of the section the contact of the ectoderm and entoderm in the mid-dorsal region is not shown in this section. That such an overgrowth as I have suggested takes place is also shown by the conditions found in the later stage itself.

Figures 56 and 57 (Plate VIII.) show that the dorsal wall of the archenteron is much more vacuolated than the more recently formed ventral wall. They also show that there has been an accumulation of the mesoderm in this ventral region, and that the cells of the ectoderm are smaller in the ventral than in the dorsal half of the embryo. All of these facts seem to point to a more rapid growth in this mid-ventral part of the embryo.

The conditions in the mid-dorsal region are of considerable interest. Assuming that the surface of contact between ectoderm and entoderm is constant now, as it has been during gastrulation, except when it is interrupted by the ephemeral cleavage cavity, we find that it no longer occupies the mid-dorsal region, but is shifted about 45° toward the anterior end of the embryo. This shifting is similar to that described by Heymons ('93) for the velar field of Umbrella, and is perhaps homologous with the shifting of the troch in the trochophore larva.

A prominent funnel-shaped opening leads into the archenteron; in the latest stages I have examined it still persists. This is the region where the anus is ultimately found, and I am therefore inclined to the view that this remnant of the posterior part of the blastopore becomes the anus of the adult; it certainly lies in the anal region.

The mouth is formed at a later period by an invagination of the ectoderm on the ventral surface of the embryo. Figure 55 (Plate VIII.) shows an embryo of a stage where the posterior opening leads into the archenteron, and the development of the head vesicle has obliterated all traces of the velar prominences. It will be seen that the ventral lip of the opening is prolonged posteriorly. This is the pedal elongation, which later becomes the podocyst. The shell gland arises on the dorsal surface as an invagination, posterior to the level of the velar region. The enlarged cells premonitory of the invagination are seen in Figure 57 (Plate VIII.).

The conclusions to which I have arrived as to the fate of the blastopore in Limax agrestis are directly contradictory to those of Fol upon L. maximus. According to him the blastopore becomes the permanent mouth. He seems, however, to have entirely overlooked the early stages in the history of the blastopore. His Figures 1 and 2 (Plate XVII.–XVIII.) represent stages in which the overgrowth of the blastopore has already taken place. The polar globules in his Figure 1 have also, it seems, been shifted somewhat, for they lie at the anterior end of the embryo. Furthermore, it is impossible to reconcile his Figures 3 and 4 with his later stages, e. g. Figure 7. It seems much more probable that what he calls the mouth in Figures 3 and 4 is the anus, and that his shell gland is in reality the oral invagination.

In concluding that the anus is formed from this persisting posterior part of the blastopore (actually or potentially), my conclusions agree with those of Bütschli ('77), Lankester ('76), and Blochmann ('83) upon Paludina. Although the anus can thus be referred to the posterior region of the blastopore, it is not so certain, owing to the peculiar manner in which the anus is formed, that the mouth corresponds to the anterior part. It seems probable that in this case the mouth must be regarded as an entirely new structure.

Cambridge, Mass., May 1, 1894.

ADDENDUM.

Since the completion of the preceding pages, Crampton's paper on *Reversal of Cleavage in a Sinistral Gasteropod* (Annals N. Y. Acad. Sci., Vol. VIII. pp. 167–169, Plate V., March, 1895) has been received. Crampton has followed the cleavage of Physa heterostropha through the twenty-four-cell stage, and a reversal of the direction of the spiral is indicated for every cleavage.

ANN ARBOR, MICH., June 24, 1895.

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EXPLANATION OF THE PLATES.

All figures were drawn from the eggs of Agriolimax agrestis, L., and are from preparations unless otherwise stated. A camera lucida was in every case employed.

A, *B*, *C*, *D*, denote the first four cleavage spheres, *A* and *B* corresponding to the anterior quadrants, *B* and *C* to the right quadrants of the embryo. For the meaning of *a*, *b*, *c*, *d*, with their exponents, consult the explanation of the system of nomenclature of cells to be found on pages 40–43.

The first, second, and third cleavage furrows are indicated by the Roman numerals I., II., III., respectively.

Arrows are used to show the common origin of the cells thus connected, the head of the arrow occupying the cell nearer the animal pole of the egg.

ABBREVIATIONS.

arch.	Archenteron.
ast'cœl.	Astrocœl.
bl'po.	Blastopore.
bl'po. a.	Anterior end of blastopore.
bl'po. p.	Posterior end of blastopore.
cav. sg.	Cleavage (or segmentation) cavity.
cnch.	Cells destined to form the shell gland.
glb. pol.	Polar globule.
pd.	Foot.
pr'c. amœ.	Amœboid process.
pr'j. vel.	Velar projections.
spa. i'cl.	Intercellular space.
spa. lns.	Lenticular space.
vac.	Vacuole.
vs. ce.	Head vesicle.

PLATE I.

Figures 1 to 7 drawn from the same living egg. × 275.

Fig. 1. Two-cell stage, beginning of flattened condition, 11.52 A. M.
Fig. 2. Same egg at 12.45 P. M. First trace of cleavage cavity.
Fig. 3. Same egg at 12.50 P. M.
Fig. 4. Same egg at 1.01 P. M.
Fig. 5. Same egg at 1.20 P. M. Maximum size of cleavage cavity.
Fig. 6. First (partial) expulsion of contents of cleavage cavity. 1.30 P. M.
Fig. 7. Same egg at 1.55 P. M. Spindles of the third generation present; the asters of the near ends of the spindles in focus.

Figures 8 to 13 drawn from another living egg of the same laying as Figures 1 to 7. × 275.

Fig. 8. At 3.45 P. M. Division into four cells completed. Typical alternate arrangement of the four blastomeres. Viewed in the direction of the *second* plane of cleavage, therefore perpendicular to the direction of Figures 1 to 7.
Fig. 9. Same egg at 3.55 P. M. Formation of a cleavage cavity.
Fig. 10. Same egg at 4.05 P. M.
Fig. 11. Maximum development of the cleavage cavity. 4.35 P. M.
Fig. 12. Same egg after a gradual total expulsion of the contents of the cavity. 4.45 P. M. Spindles of the fourth generation present.
Fig. 13. Formation of the eight cells of the fourth generation. Persistence of the cleavage cavity. 5.45 P. M.
Fig. 14. Lateral view of two-cell stage. First cleavage plane just completed. Astrocœls present. × 490.

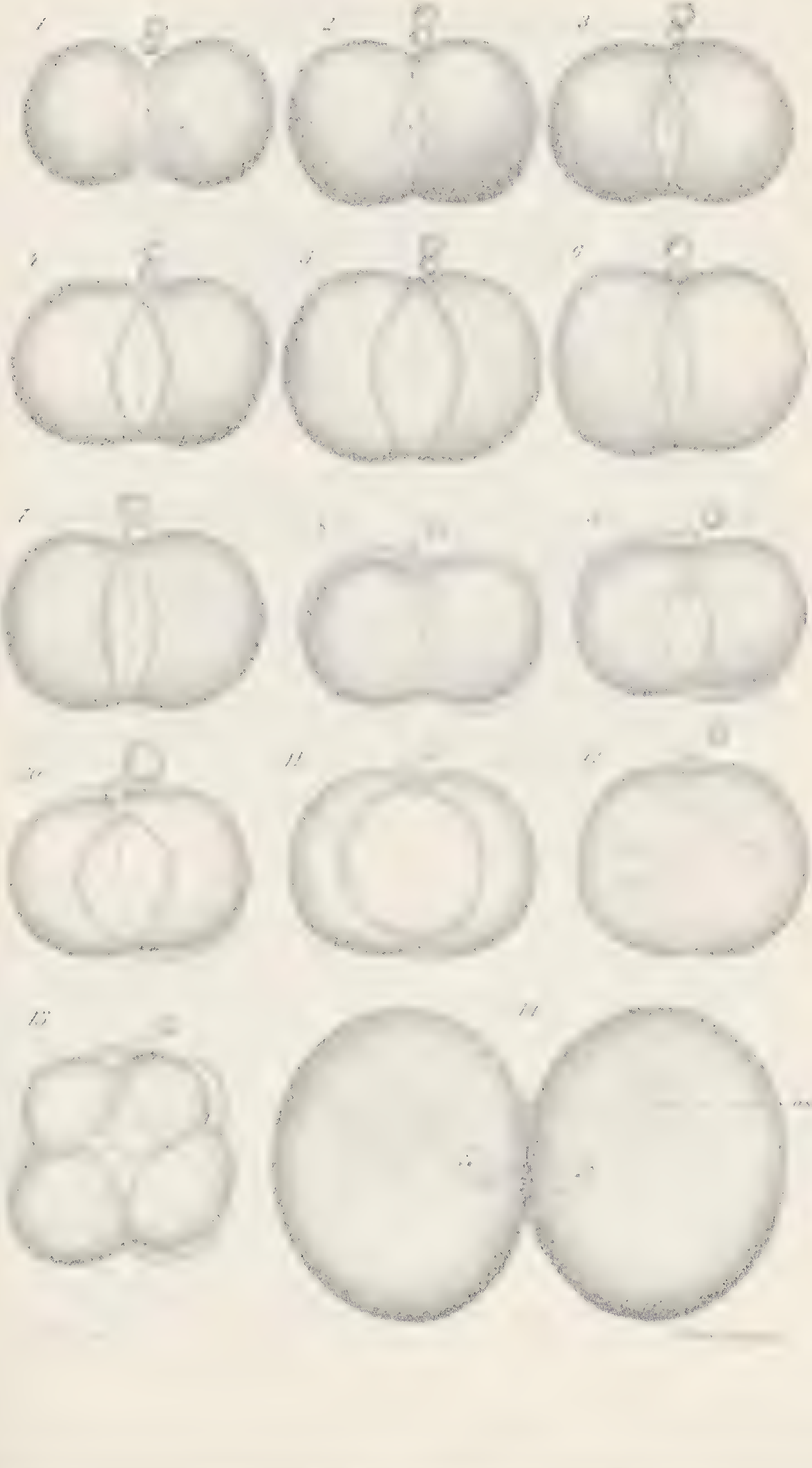


PLATE II.

All figures magnified 490 diameters.

Fig. 15. Two-cell stage from animal pole. Spindles of the third generation present. No cleavage cavity. Deeper ends of the spindles (asters of *B* and *D*) shown by lighter lines.

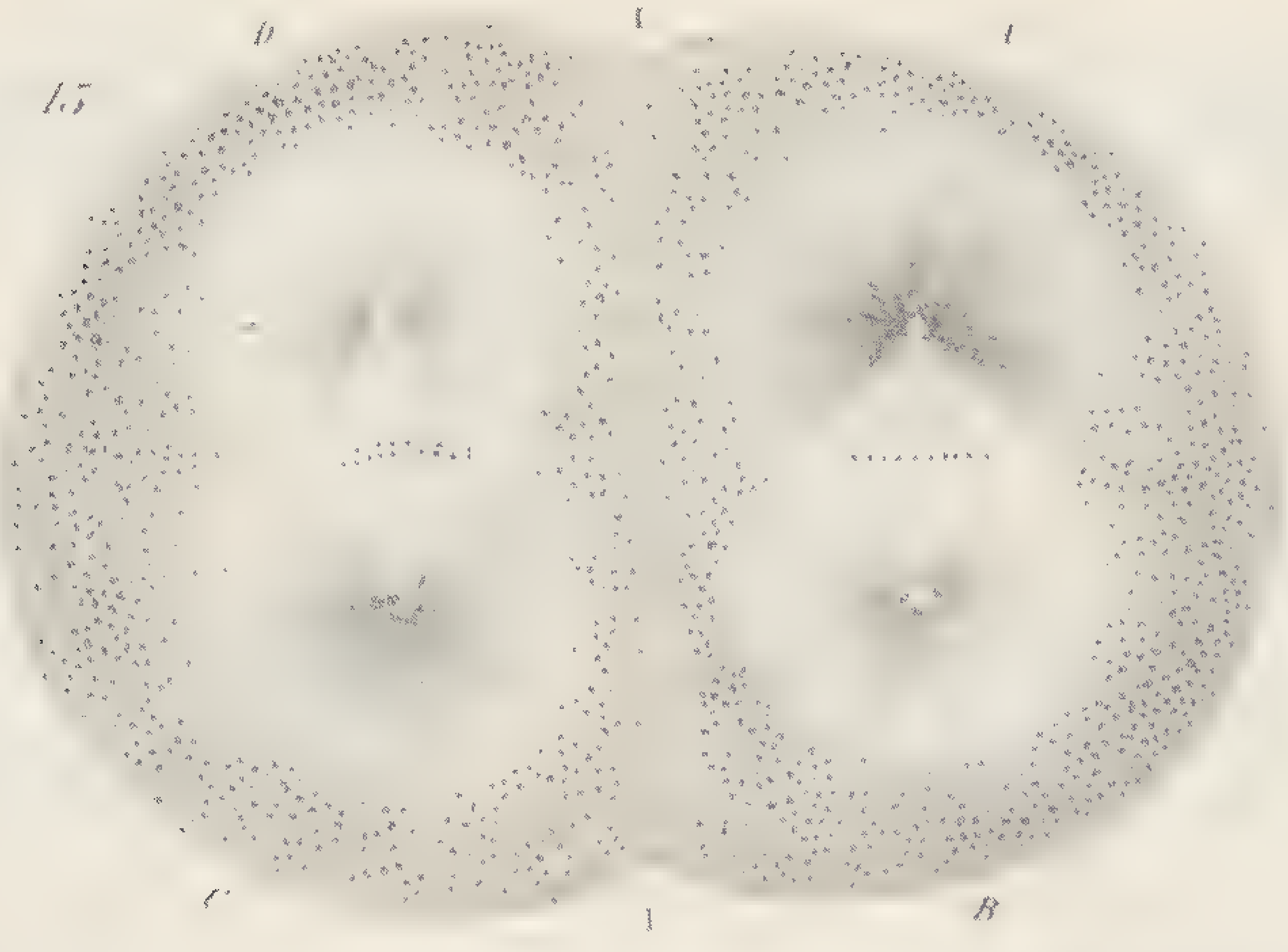
Fig. 16. Four-cell stage from the animal pole. Second cleavage furrow almost completed. Cleavage cavity and lenticular spaces present.

Fig. 17. Four-cell stage from the animal pole. Cleavage cavity present. Spindles of the fourth generation.

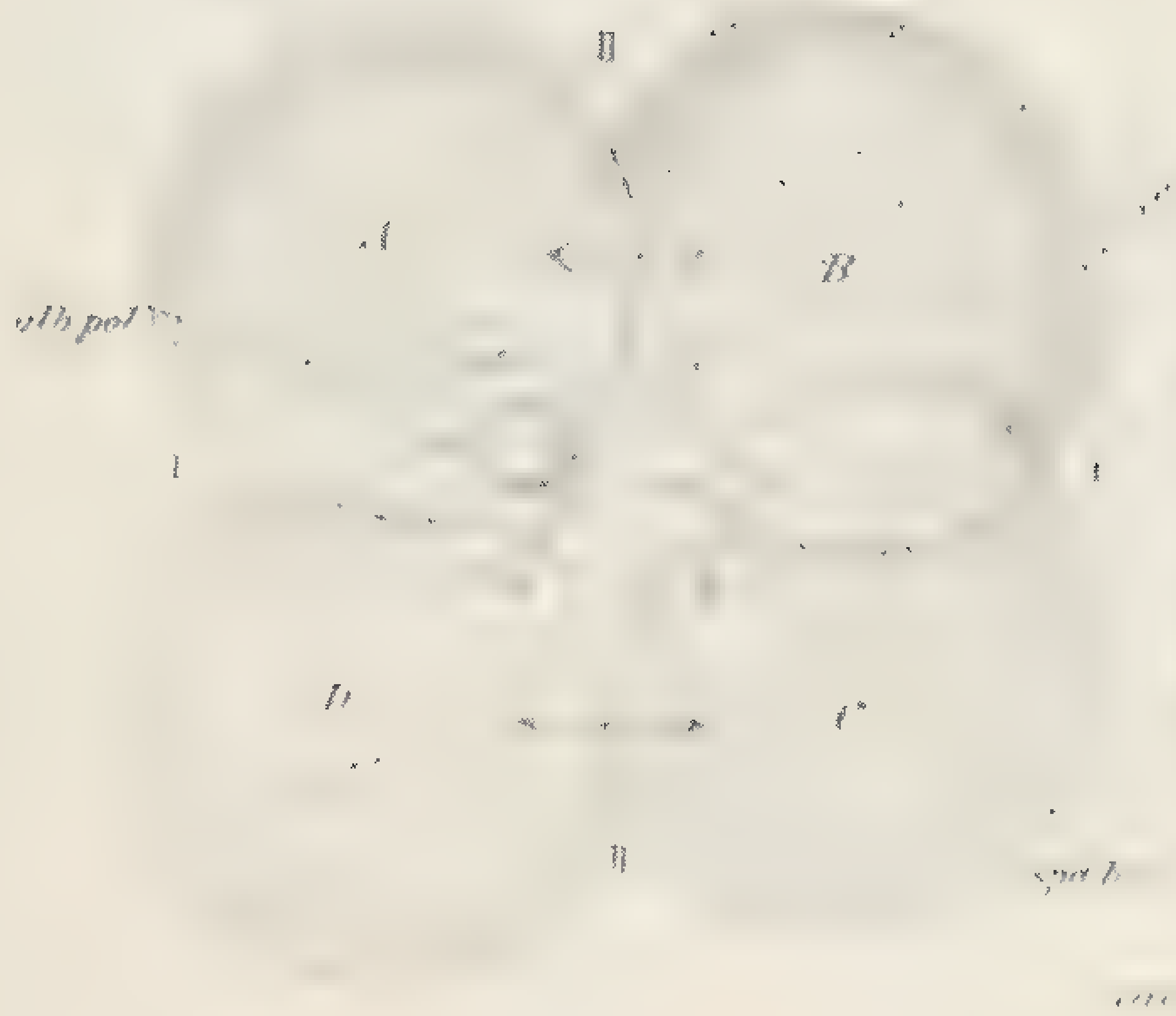
Fig. 18. Same egg from the anterior end.

Fig. 19. Eight-cell stage from the animal pole, composed of the two quartets of the fourth generation, $a^{4\cdot1}-d^{4\cdot1}$ and $a^{4\cdot2}-d^{4\cdot2}$. Third cleavage plane just completed. Astrocœls present.

15


D
I
A
C
I
B

16


II
A
B
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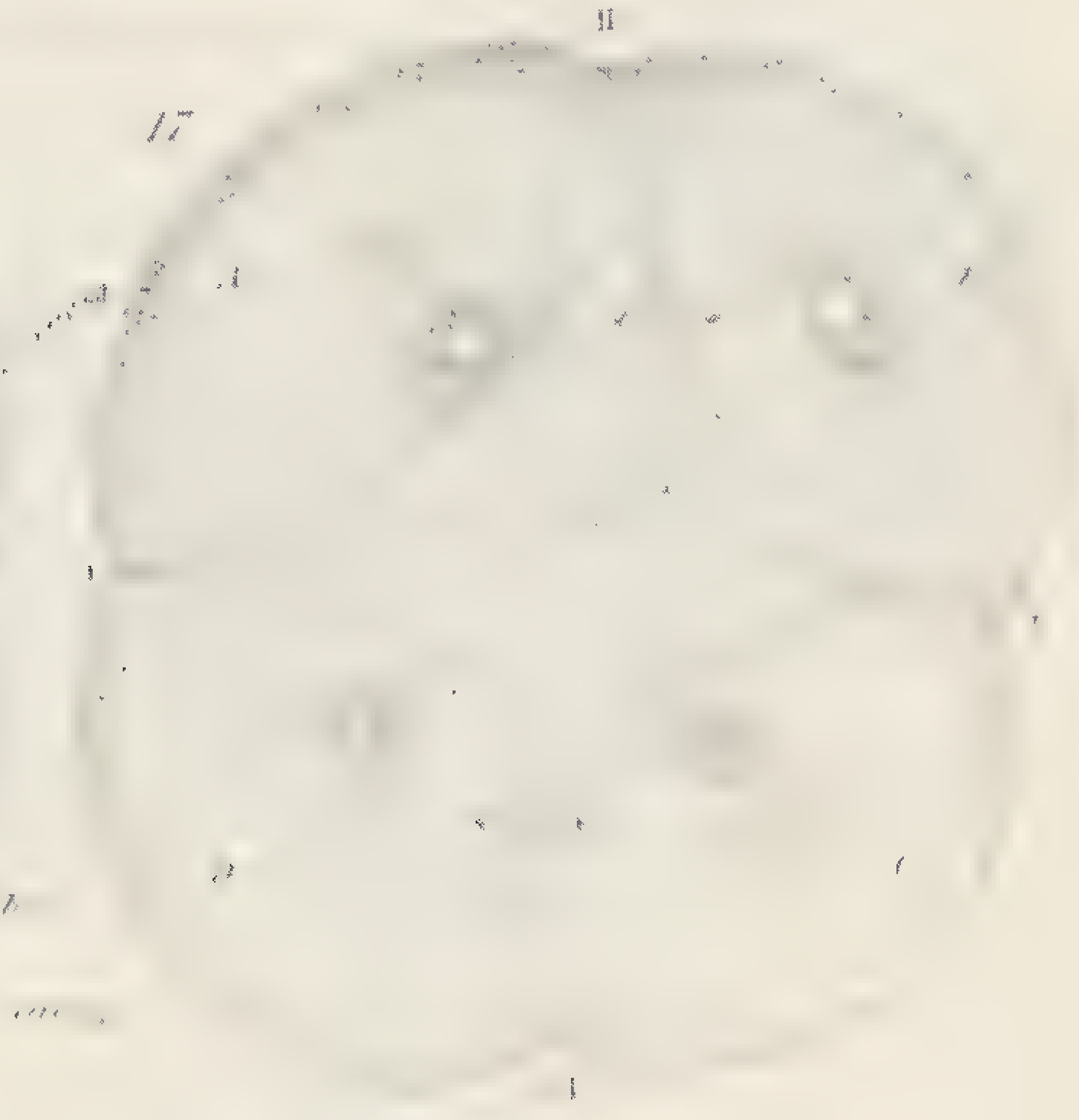
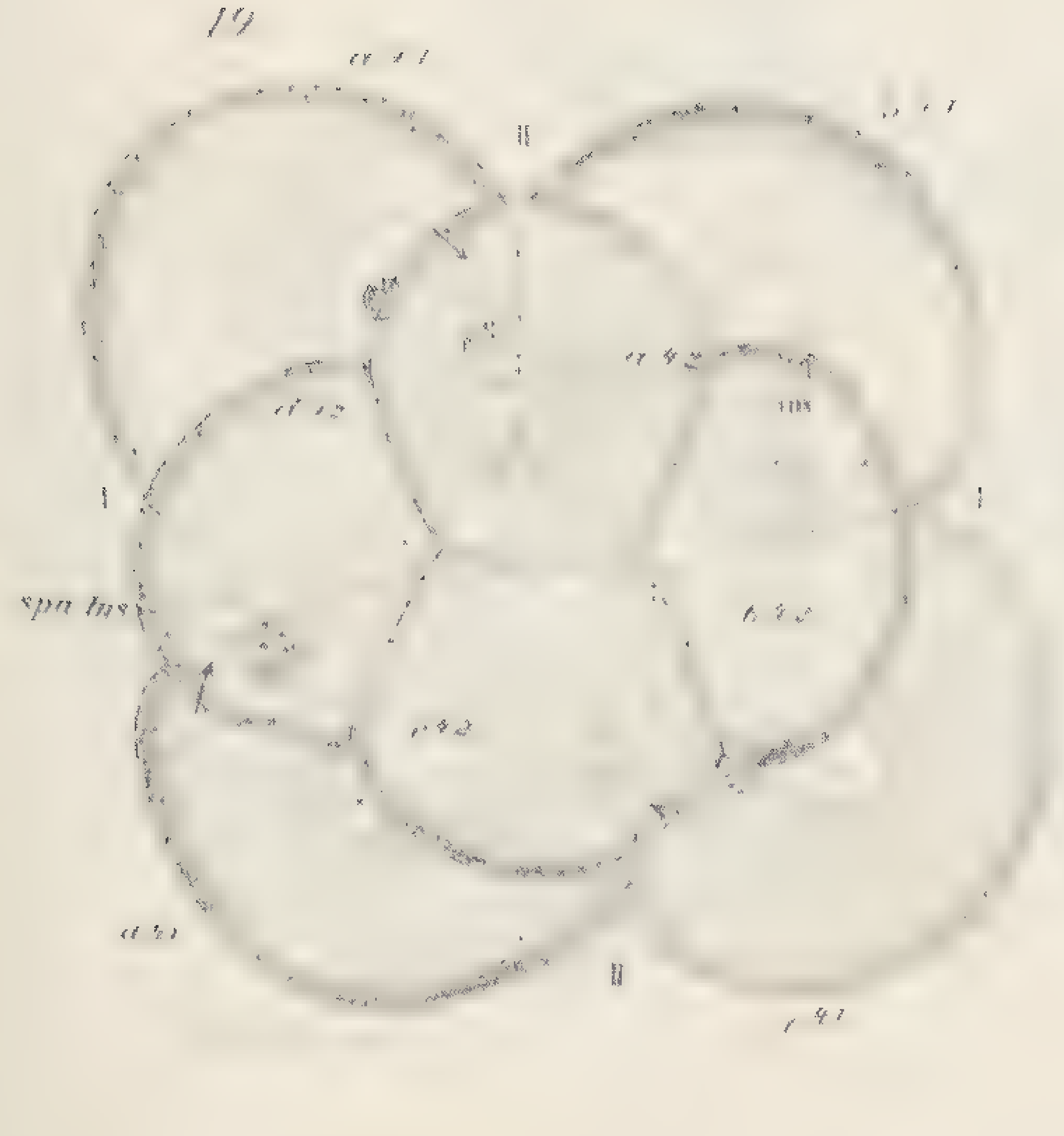
I
D
C
II
spa b

17


II
A
B
I
D
C
I

19


a 4 1
b 4 1
II
a 4 2
I
I
spa lms
b 4 2
c 4 2
d 4 1
II
c 4 1

18

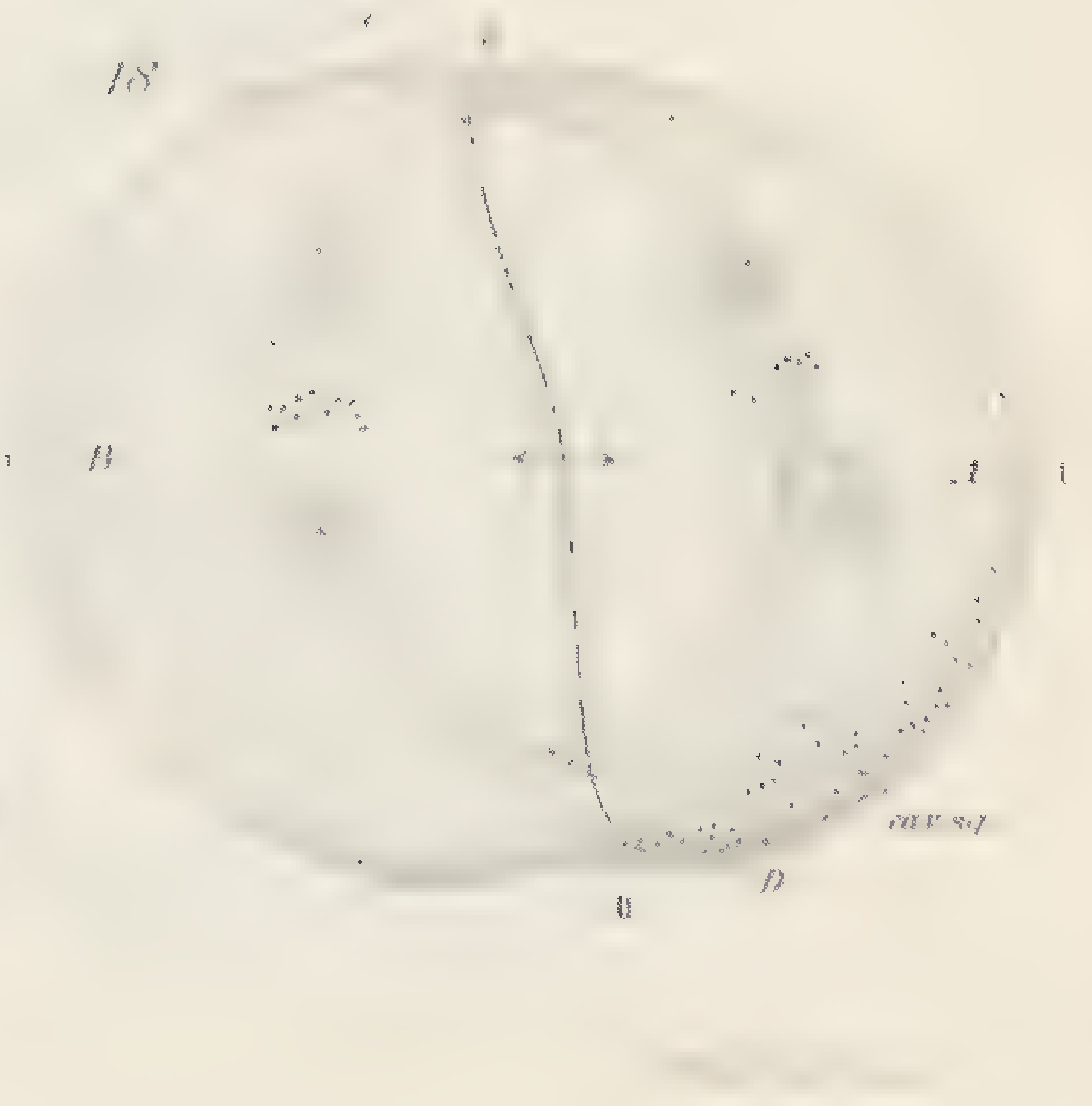

B
I
A
I
D
II

PLATE III.

All figures magnified 490 diameters.

Fig. 20. Eight-cell stage from the anterior end. Cleavage cavity and lenticular spaces present. Spindles of the fifth generation.

Fig. 21. View of the same egg from the animal pole.

Fig. 22. Sixteen-cell stage viewed somewhat obliquely from the right anterior quadrant, composed of the following cells: $a^{5\cdot1}-d^{5\cdot1}$; $a^{5\cdot2}-d^{5\cdot2}$; $a^{5\cdot3}-d^{5\cdot3}$; and $a^{5\cdot4}-d^{5\cdot4}$. Cleavage cavity present.

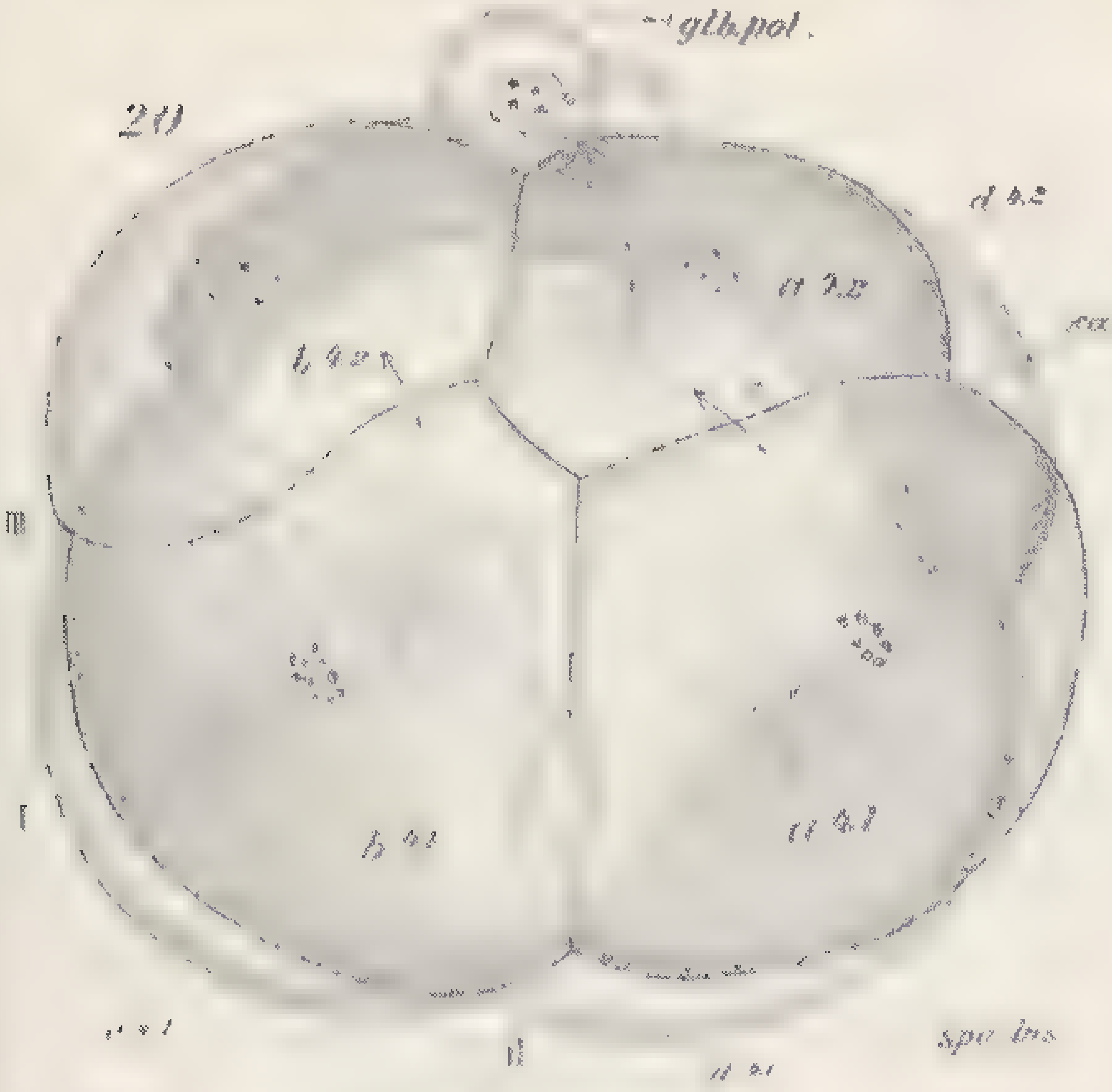
Fig. 23. Lateral view of twenty-four-cell stage. Recent division of quartets resulting in 6.1, 6.2, 6.3, 6.4. Vacuolation at the animal pole.

Fig. 24. Oblique section of same egg. Fifth section in a series of twelve. Intercellular spaces at the animal pole. Cleavage cavity present. x, longitudinal section of intercellular space.

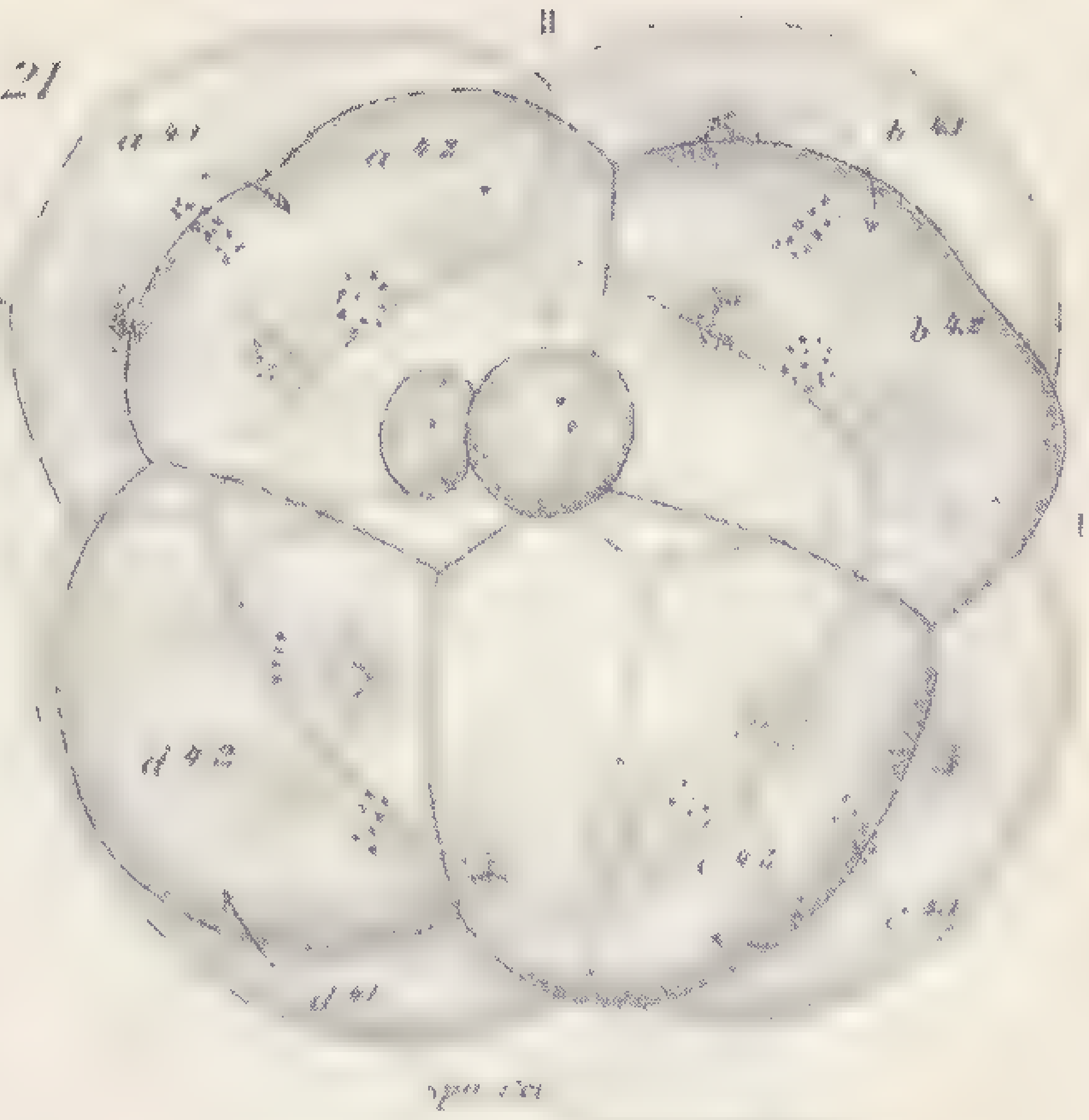
Fig. 25. Seventh section in same series.

Fig. 26. Transverse section of an embryo of about one hundred cells, showing vacuolated condition of all three germ layers.

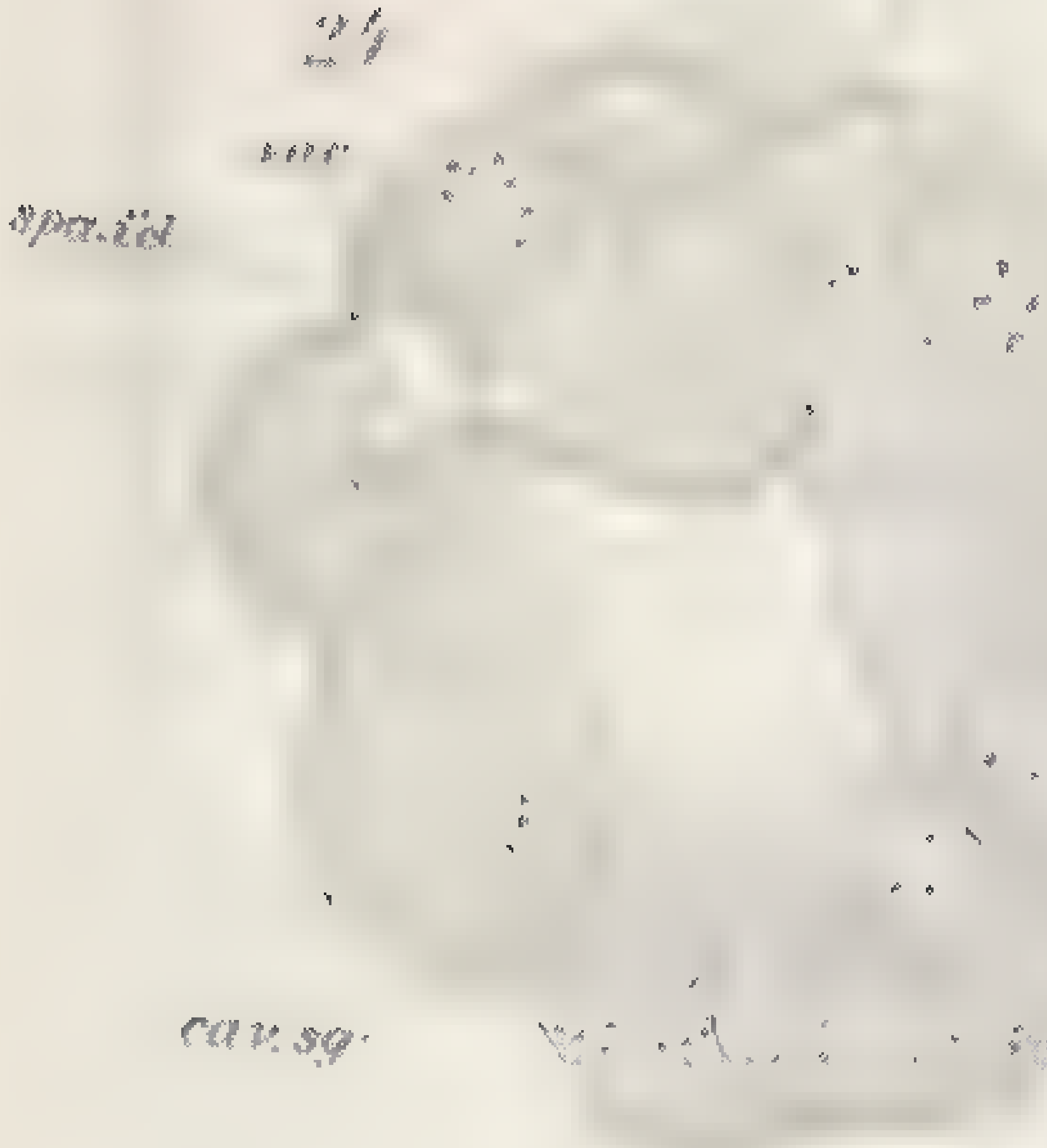
20


glb.pol.
d 4.2
a 4.2
b 4.2
III
I
b 4.1
a 4.1
c 4.1
II
d 4.1
spa.ins.

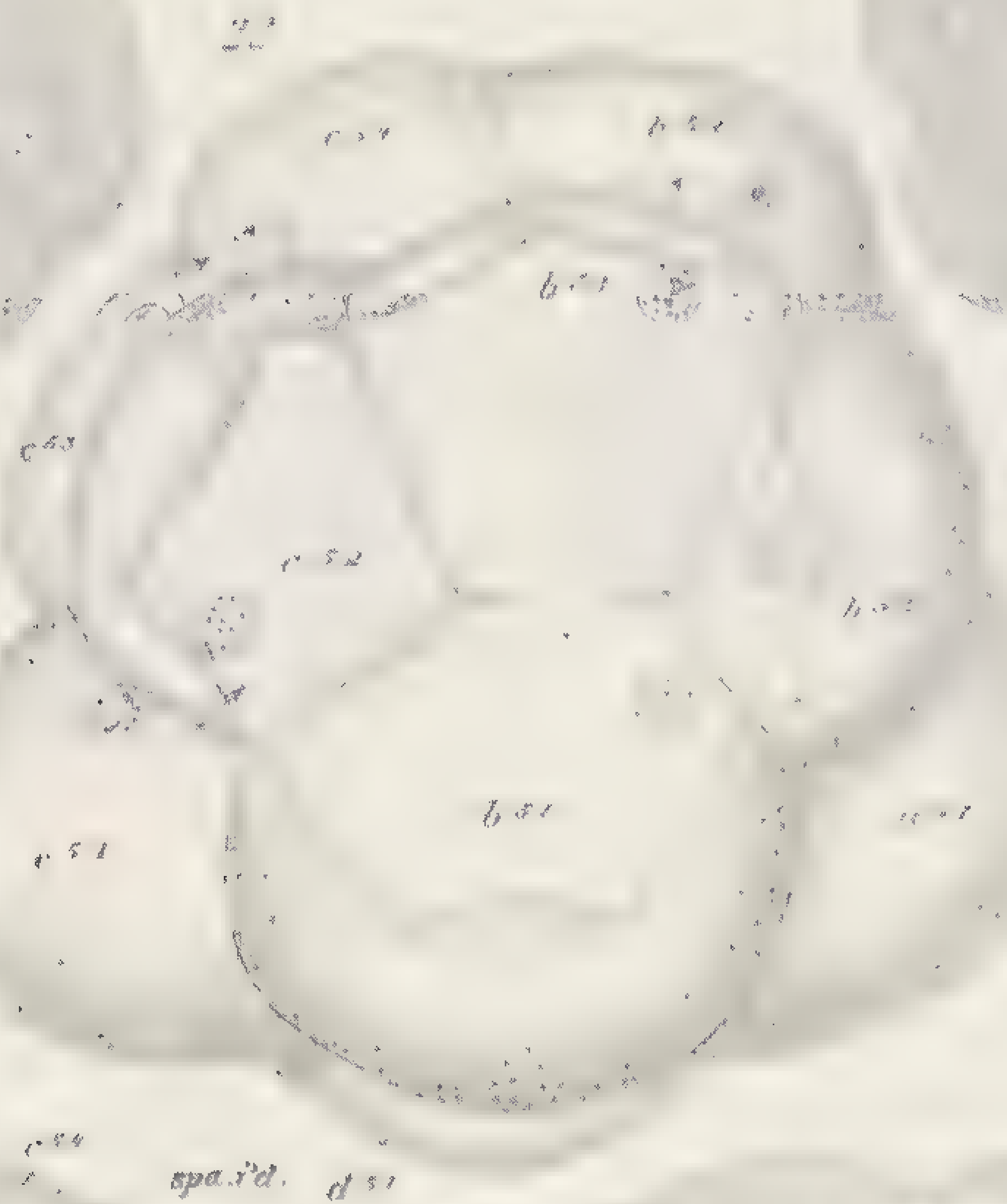
21


II
a 4.1
a 4.2
b 4.1
cav.sg.
b 4.2
I
d 4.2
c 4.2
d 4.1
c 4.1
spa.vt.

24


suc.
spa.vd.
cav.sg.
spa.vt.

22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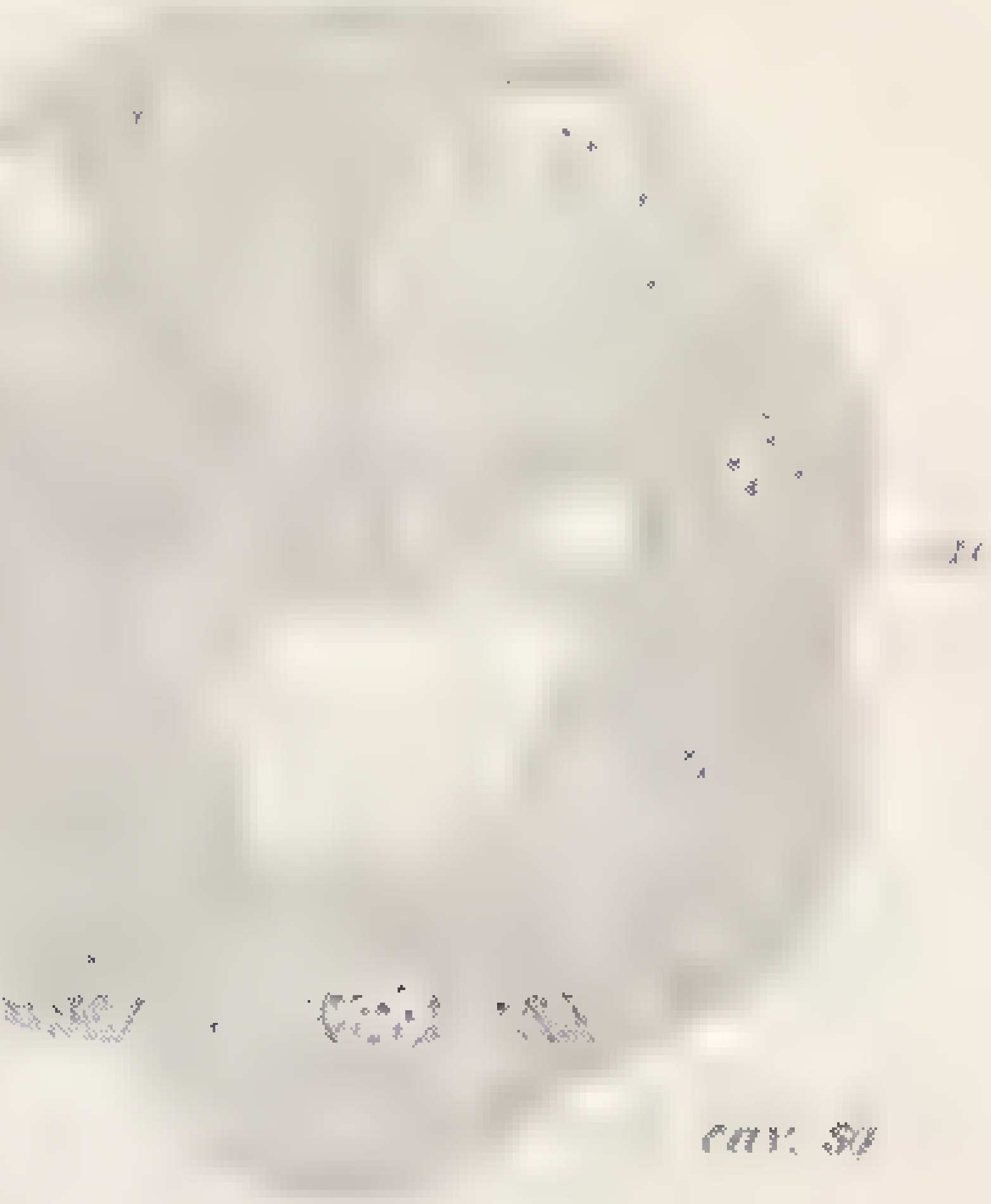
b 5.1
b 5.1
c 5.3
c 5.2
b 5.2
c 5.1
b 5.1
a 5.1
cav.sg.
spa.3'd.
d 5.1

25



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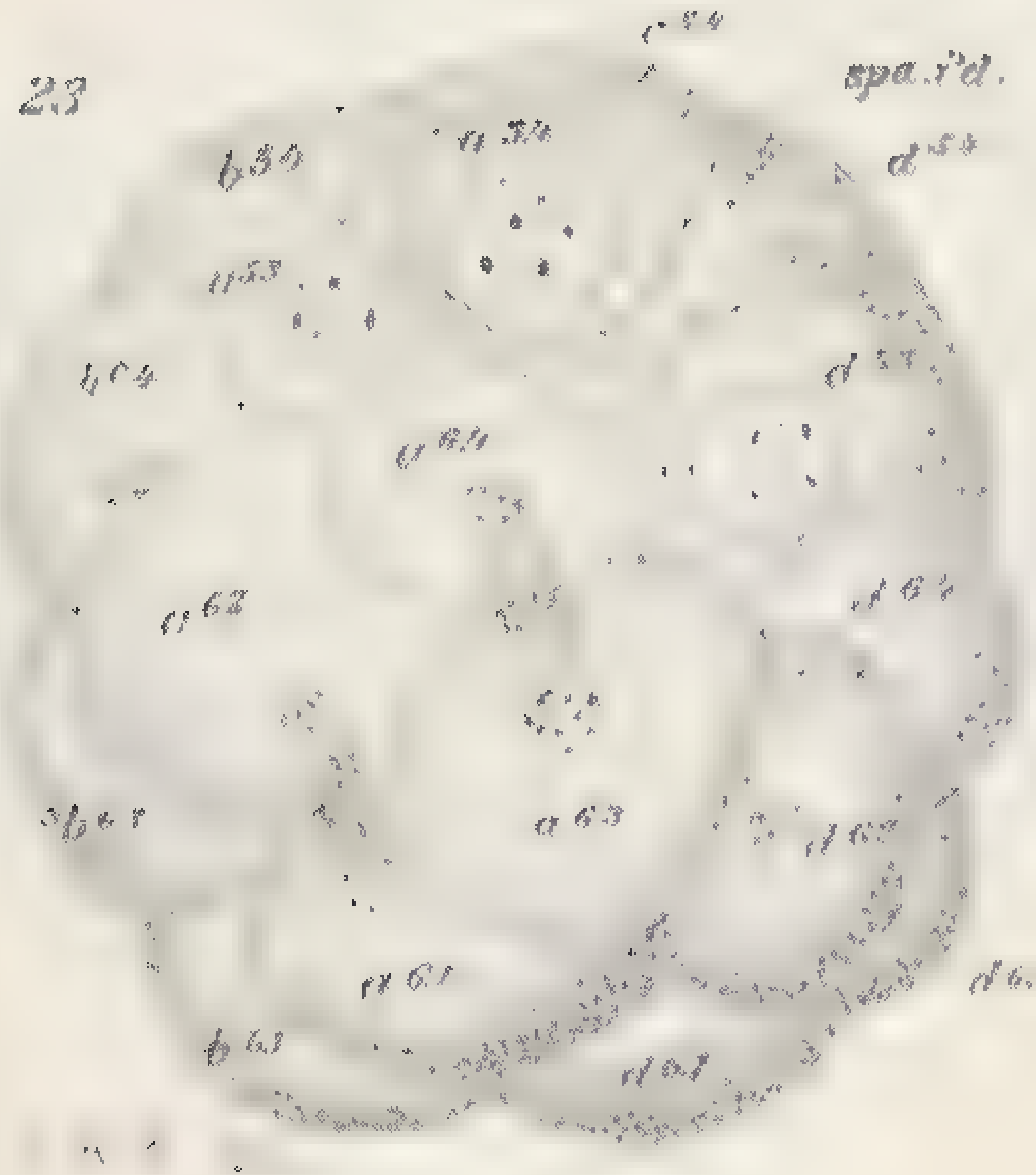

c 5.4
b 5.4
a 5.4
d 5.4
a 5.3
b 6.4
d 5.3
a 6.4
a 6.2
d 6.4
b 6.1
a 6.3
d 6.2
a 6.1
d 6.3
b 6.3
d 6.1

26

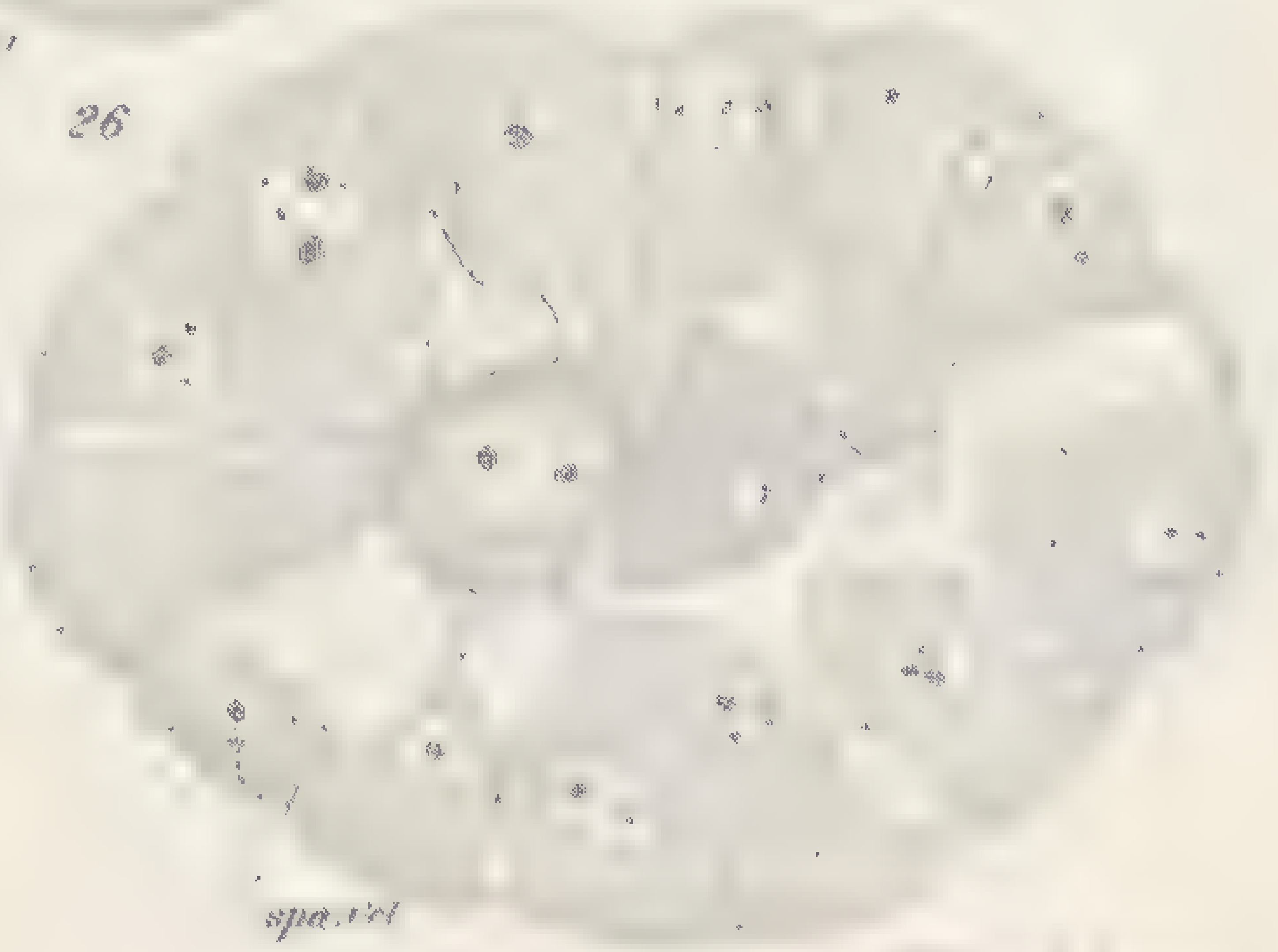


PLATE IV.

All figures magnified 490 diameters.

Figs. 27-30. Twenty-four-cell stage, composed of the following cells: $a^{5 \cdot 4} - d^{5 \cdot 4}$; $a^{5 \cdot 3} - d^{5 \cdot 3}$; $a^{6 \cdot 4} - d^{6 \cdot 4}$; $a^{6 \cdot 3} - d^{6 \cdot 3}$; $a^{6 \cdot 2} - d^{6 \cdot 2}$; $a^{6 \cdot 1} - d^{6 \cdot 1}$.

Fig. 27. From the vegetative pole.

Fig. 28. From the animal pole, showing vacuolation.

Fig. 29. View of the right posterior quadrant (*c*). Vacuolation of the animal pole.

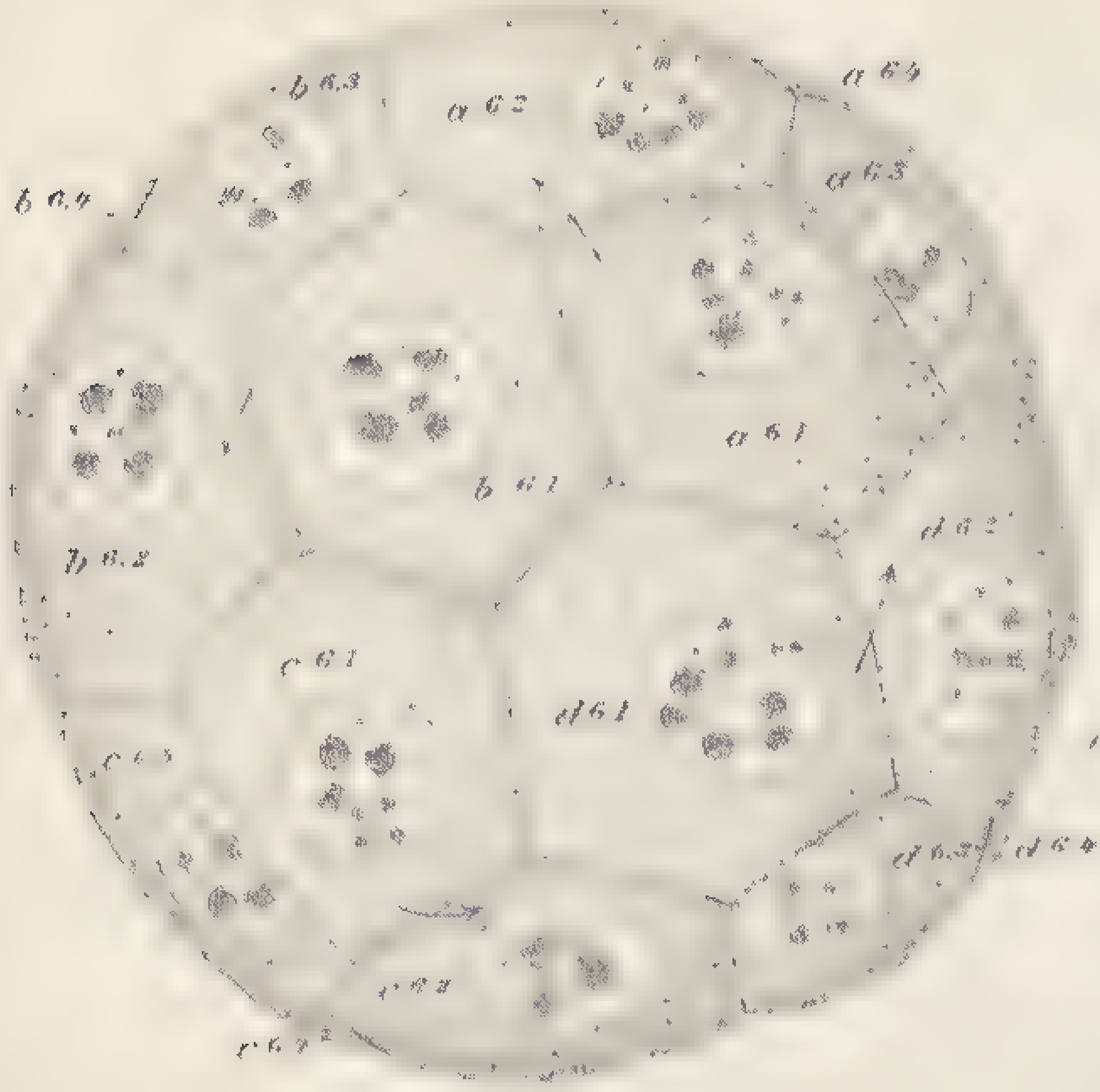
Fig. 30. Optical section along vertical axis cutting the quadrants *b* and *d*.

Figs. 31, 32. Twenty-four-cell stage, similar to the egg shown in Figures 27–30. Division of the quartet 6.2.

Fig. 31. View of the anterior end. Spindle in $b^{6 \cdot 2}$. Polar globule present.

Fig. 32. Same egg from the vegetative pole. Spindles in $b^{6 \cdot 2}$, $c^{6 \cdot 2}$, $d^{6 \cdot 2}$.

27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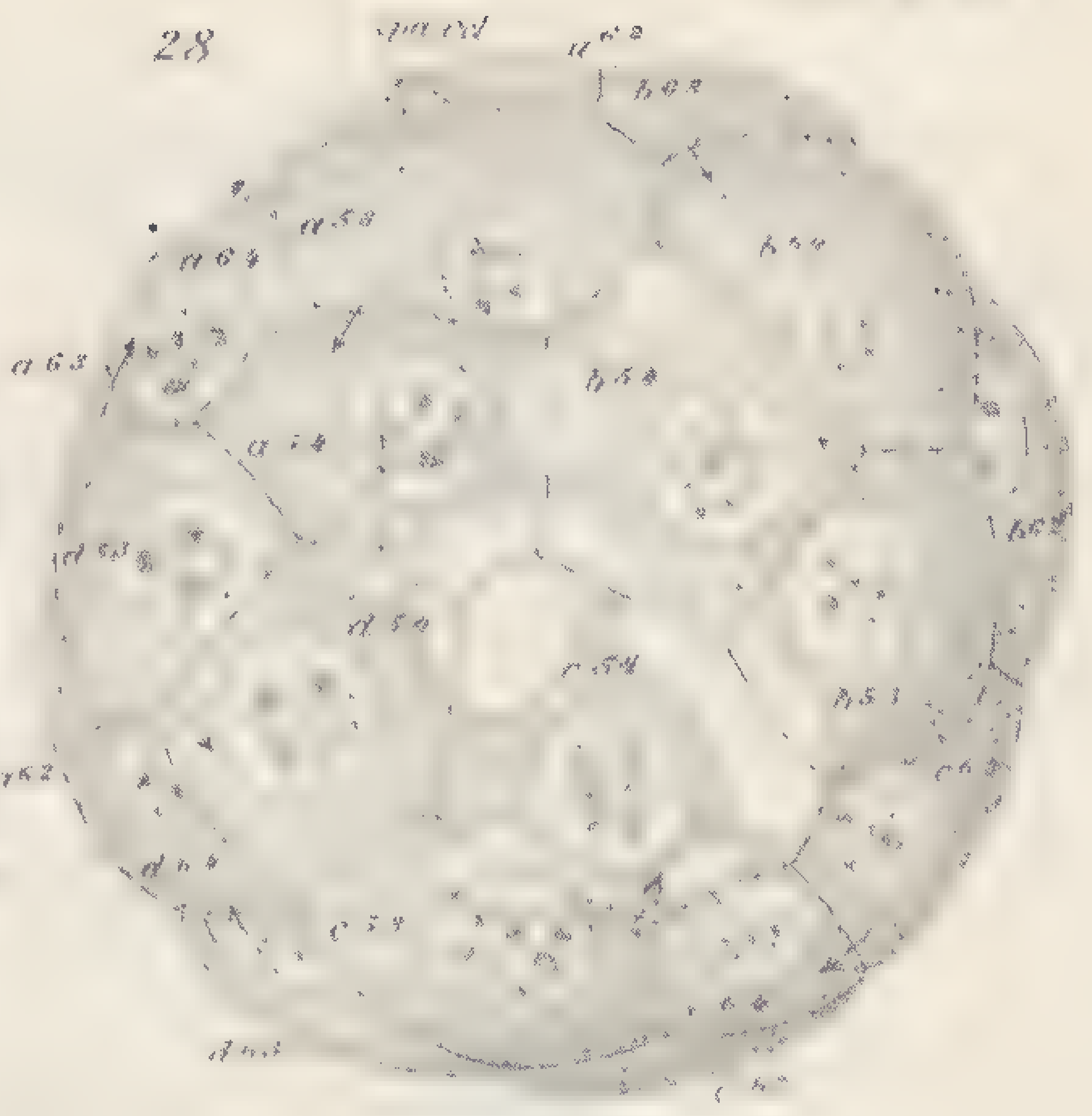
a 6.4
b 6.4
a 6.3
a 6.1
b 6.1
b 6.2
d 6.2
c 6.1
d 6.1
c 6.3
a 6.2
d 6.3
d 6.4
c 6.2
c 6.4

28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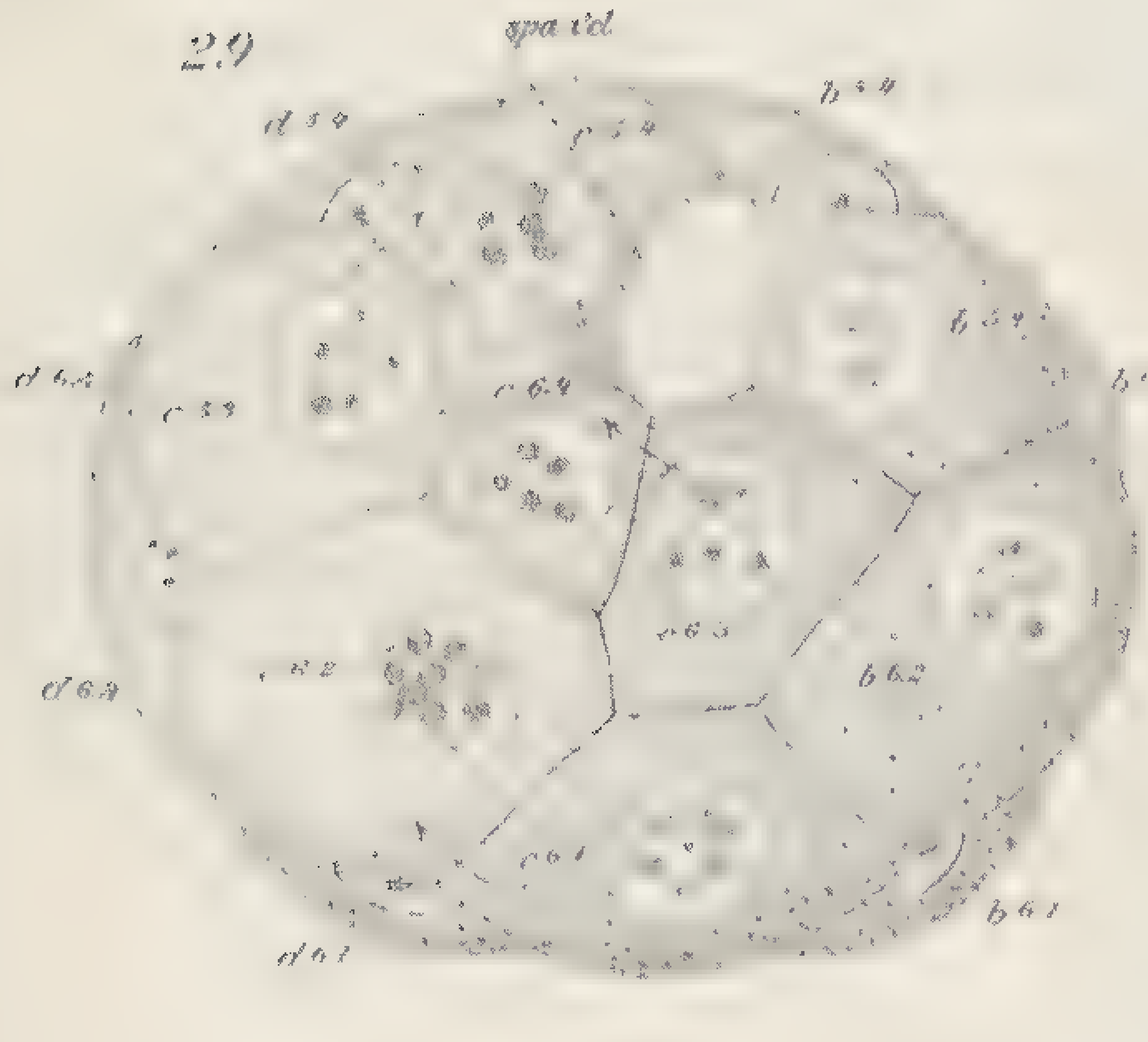
b 6.2
a 5.3
a 6.4
b 5.4
a 6.3
b 5.4
a 5.4
d 6.3
b 6.2
d 5.4
c 5.4
b 5.3
a 6.2
c 6.3
d 6.4
c 5.4
d 6.3
c 6.2

29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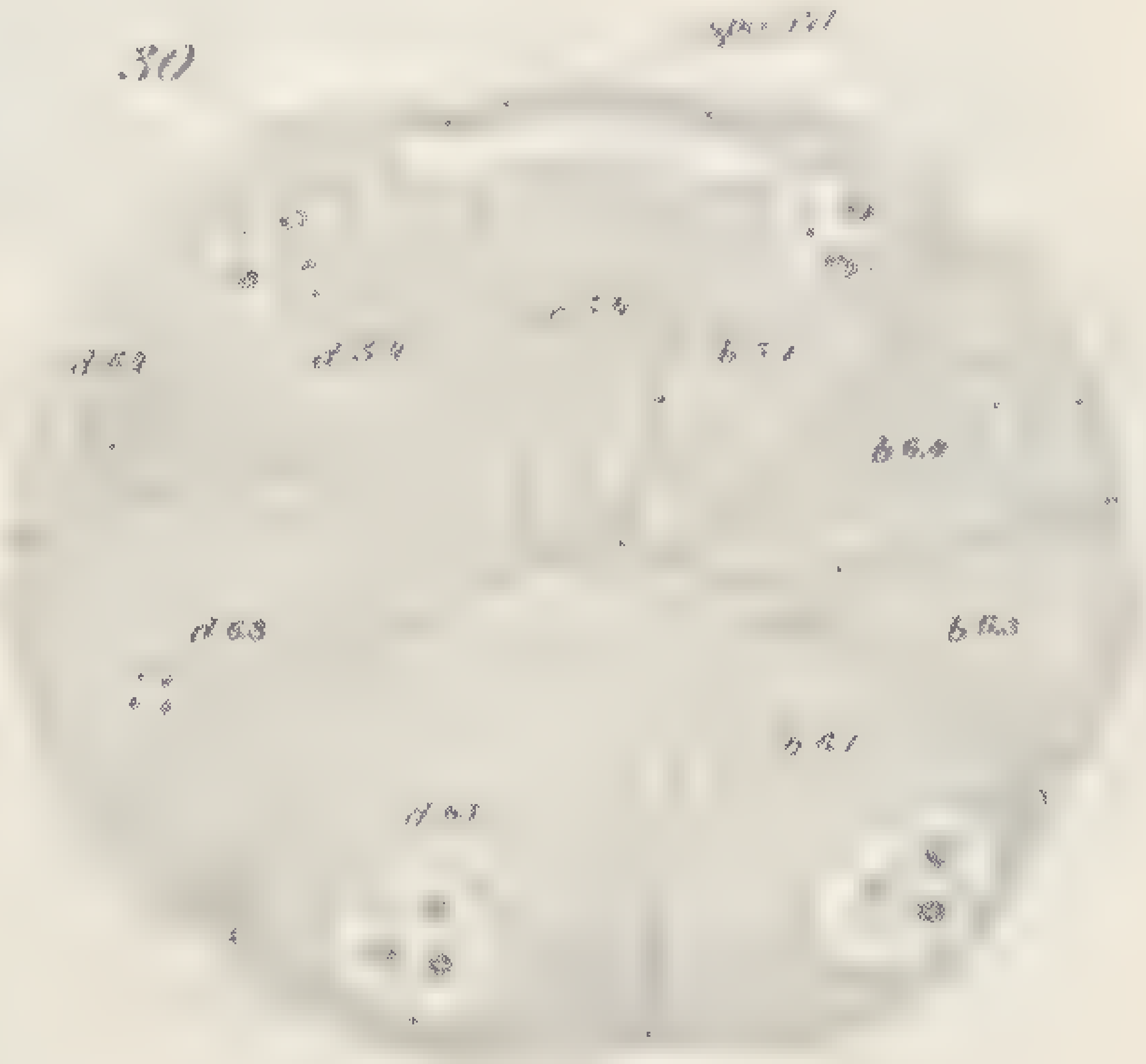
c 6.3
d 6.3
c 6.2
b 6.2
c 6.1
b 6.1
d 6.1

30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d 5.4
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d 6.3
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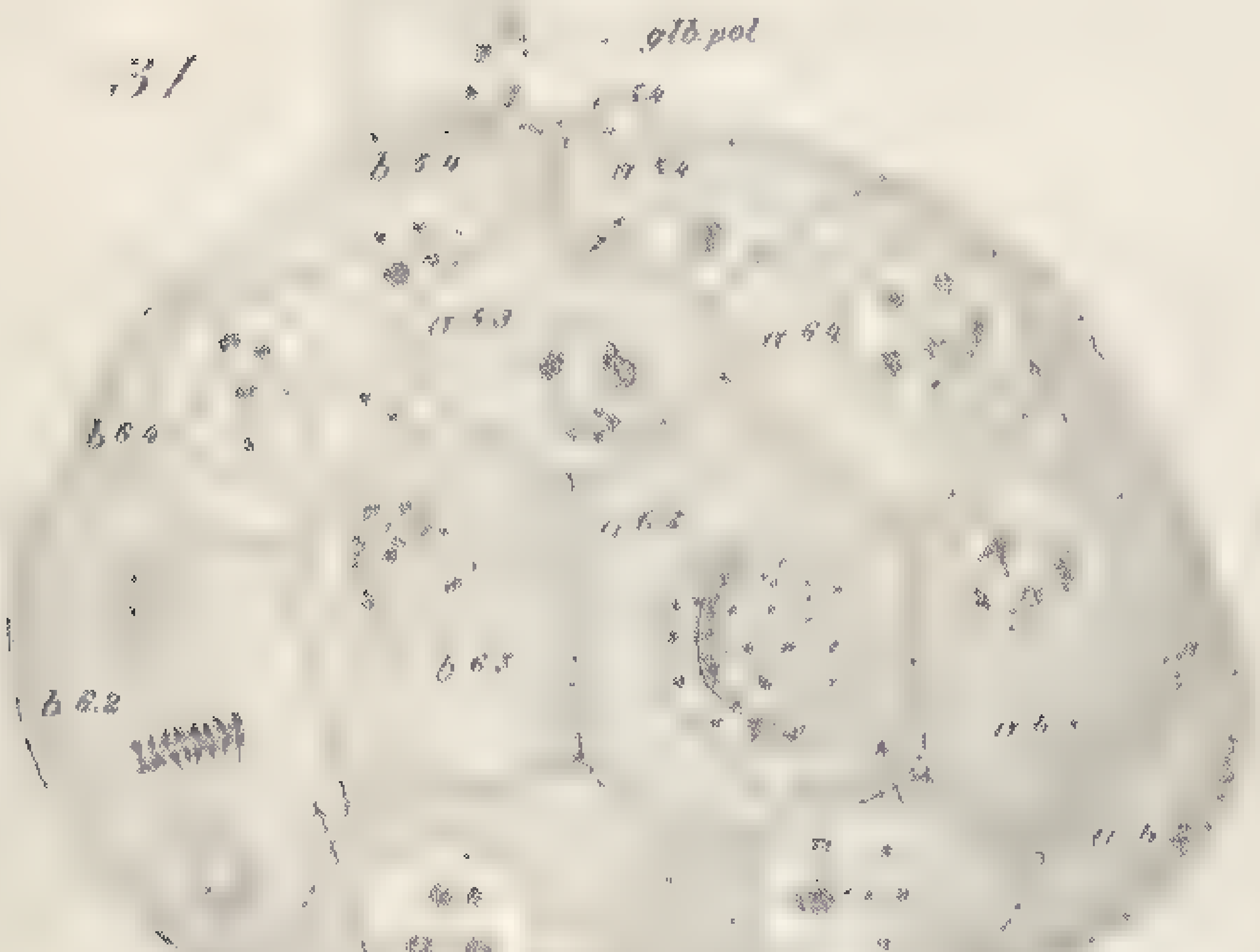
b 6.1
d 6.1

31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b 6.4
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b 6.3
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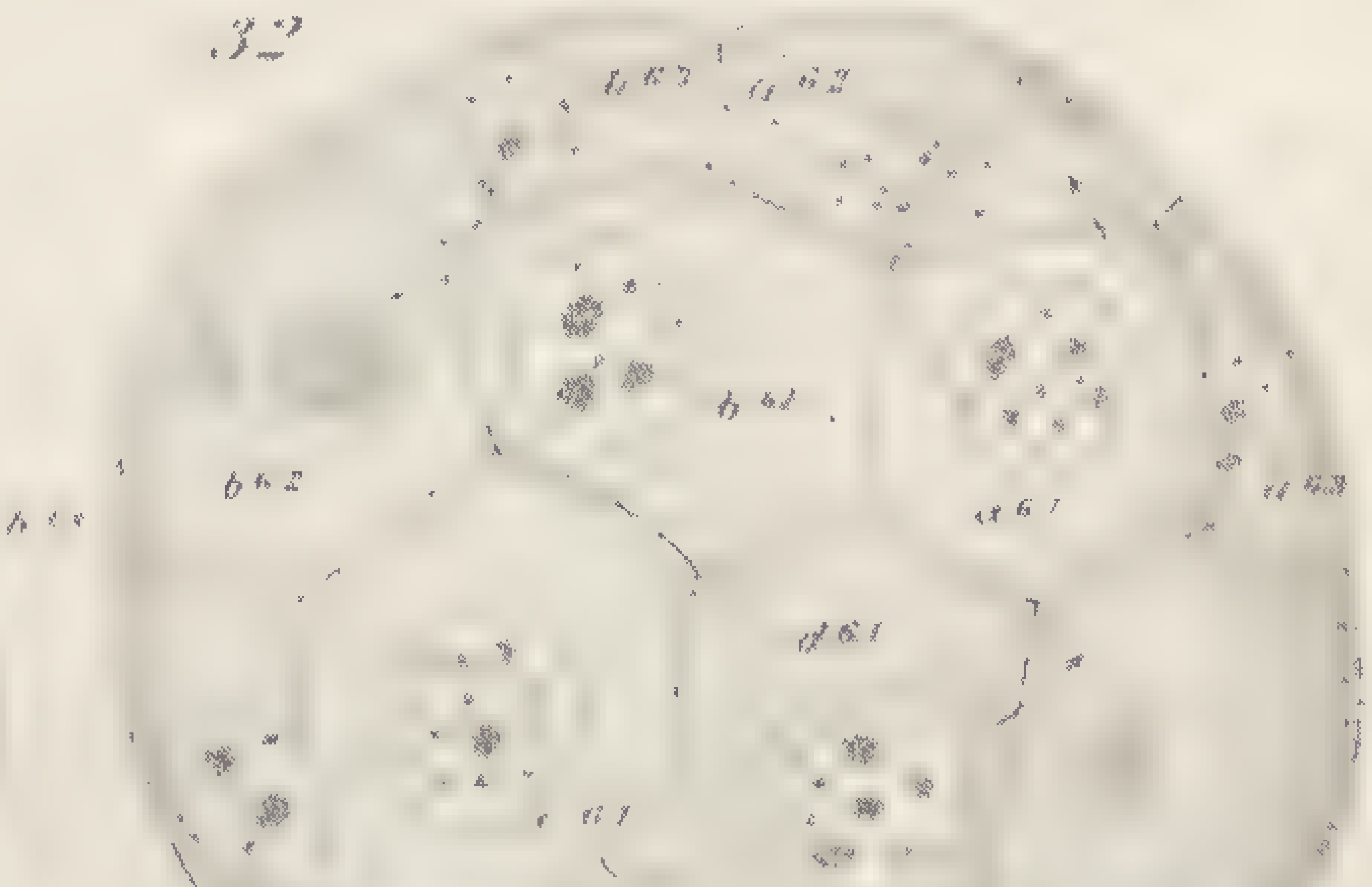
a 6.1
c 6.1
d 6.1

32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b 6.1
b 6.2
a 6.3
a 6.1
d 6.1
c 6.1
c 6.3
d 6.2
c 6.2
d 6.3
c 6.4
d 6.4

PLATE V.

Figs. 33, 34. Twenty-four-cell stage, composed of the following cells: $a^{5\cdot 4}-d^{5\cdot 4}$; $a^{5\cdot 3}-d^{5\cdot 3}$; $a^{6\cdot 4}-d^{6\cdot 4}$; $a^{6\cdot 3}-d^{6\cdot 3}$; $a^{6\cdot 2}-d^{6\cdot 2}$; $a^{6\cdot 1}-d^{6\cdot 1}$.

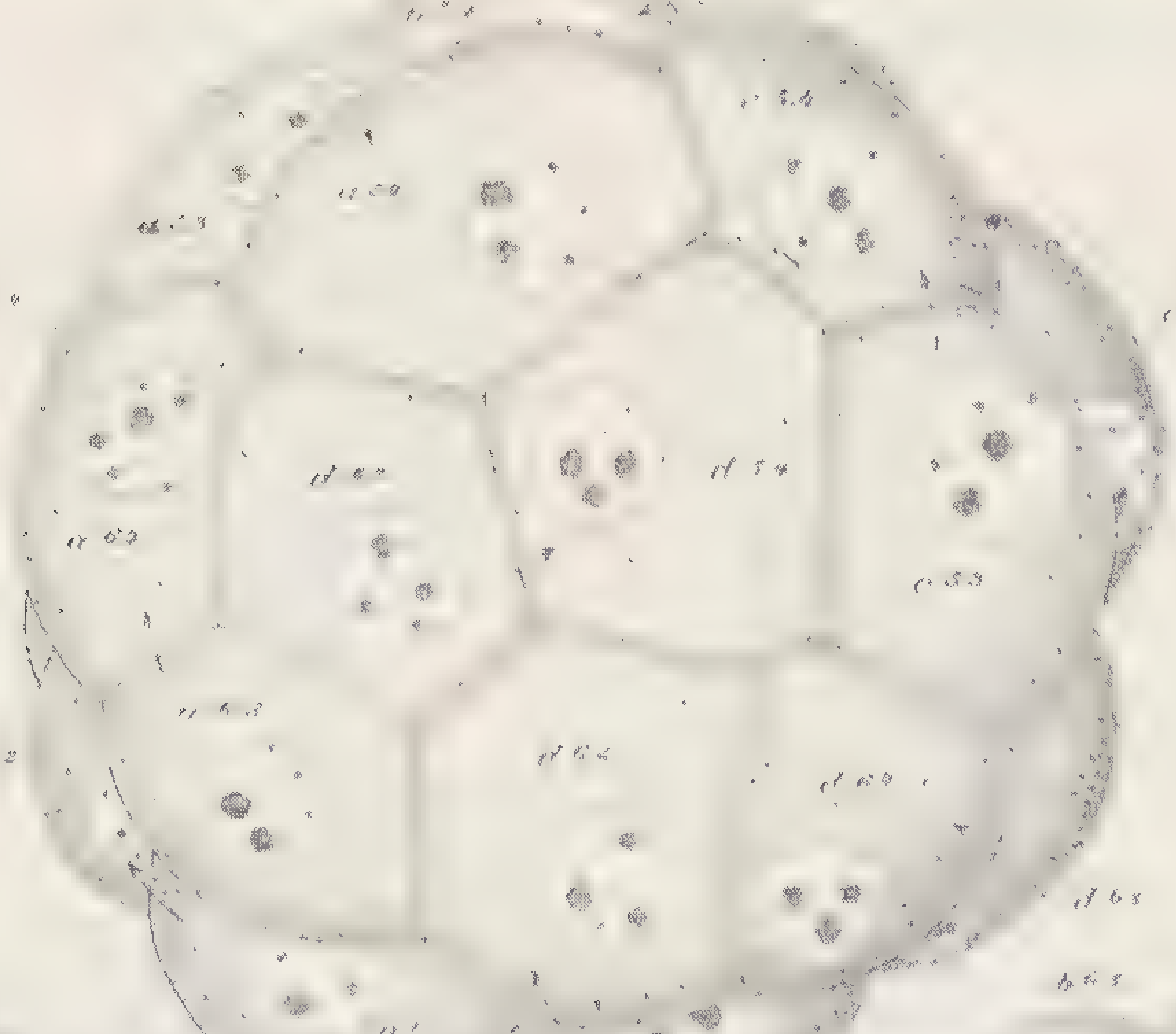
Fig. 33. View of the left posterior quadrant (*d*) from below. × 490.

Fig. 34. Optical section of egg shown in the preceding figure, and in the plane of the paper, showing the right anterior hemisphere from the inside. Large cleavage cavity present. Amœboid process (*pr'c. amœ.*) on the cell $a^{6\cdot 2}$. × 490.

Fig. 35. Twenty-eight-cell, stage composed of the following cells: — $a^{5\cdot 4}$ and $\frac{b^{6\cdot 8}}{b^{6\cdot 7}}$, $\frac{c^{6\cdot 8}}{c^{6\cdot 7}}$, $\frac{d^{6\cdot 8}}{d^{6\cdot 7}}$; $a^{5\cdot 3}-d^{5\cdot 3}$; $a^{6\cdot 4}-d^{6\cdot 4}$; $a^{6\cdot 3}-d^{6\cdot 3}$; $a^{6\cdot 2}$, $b^{6\cdot 2}$, $\frac{c^{7\cdot 4}}{c^{7\cdot 3}}$, $d^{6\cdot 2}$; $a^{6\cdot 1}-d^{6\cdot 1}$. View from the animal pole. Division of the quartets 5.4 and 6.2. × 510.

Fig. 36. Twenty-five-cell stage, composed of $a^{5\cdot 4}$, $\frac{b^{6\cdot 8}}{b^{6\cdot 7}}$, $c^{5\cdot 4}$, $d^{5\cdot 4}$; $a^{5\cdot 3}-d^{5\cdot 3}$; $a^{6\cdot 4}-d^{6\cdot 4}$; $a^{6\cdot 3}-d^{6\cdot 3}$; $a^{6\cdot 2}-d^{6\cdot 2}$; $a^{6\cdot 1}-d^{6\cdot 1}$. View of the animal pole. Division of $a^{5\cdot 4}-d^{5\cdot 4}$. Spindles in $c^{5\cdot 3}$, $c^{6\cdot 4}$. × 510.

33



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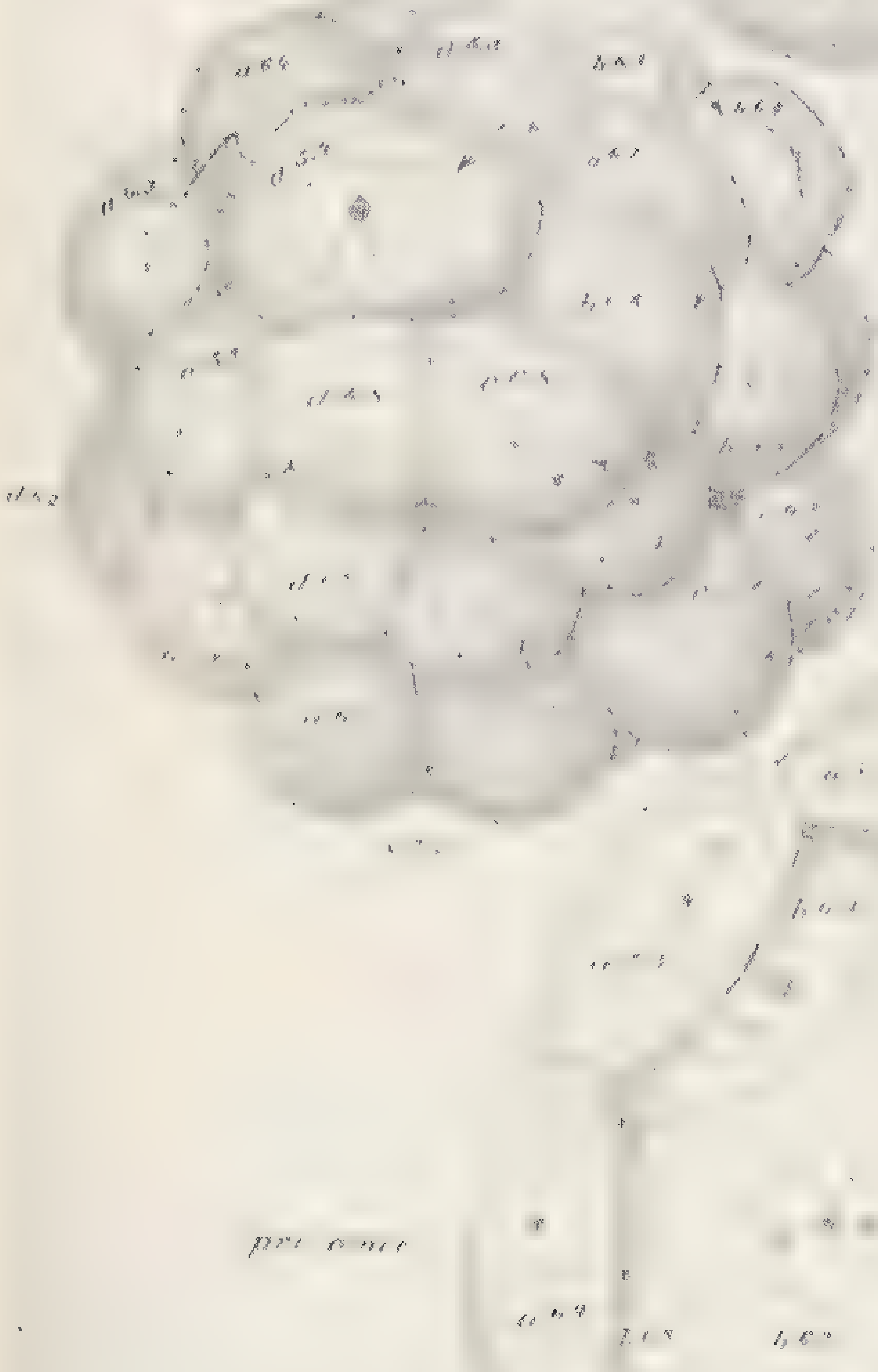

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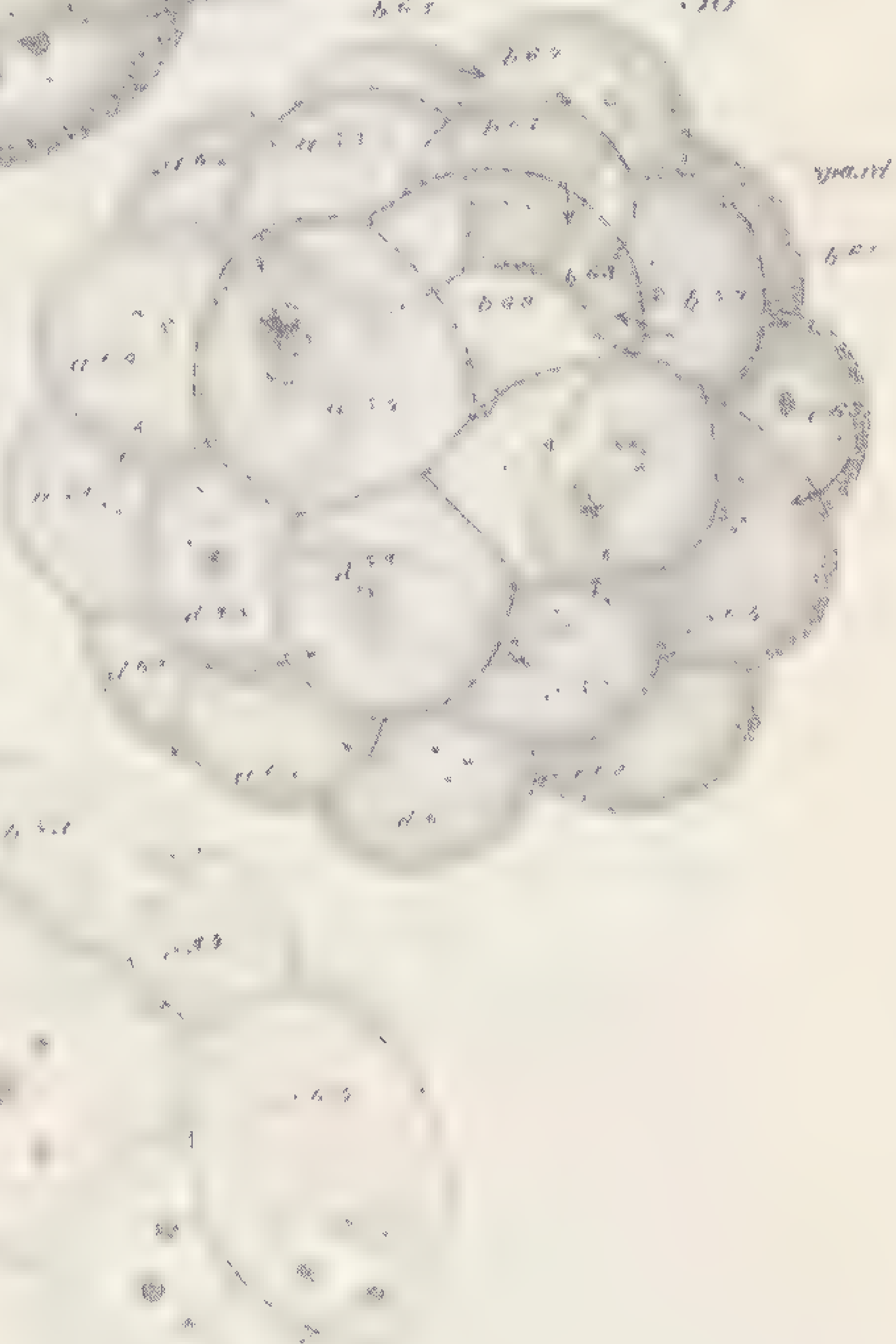

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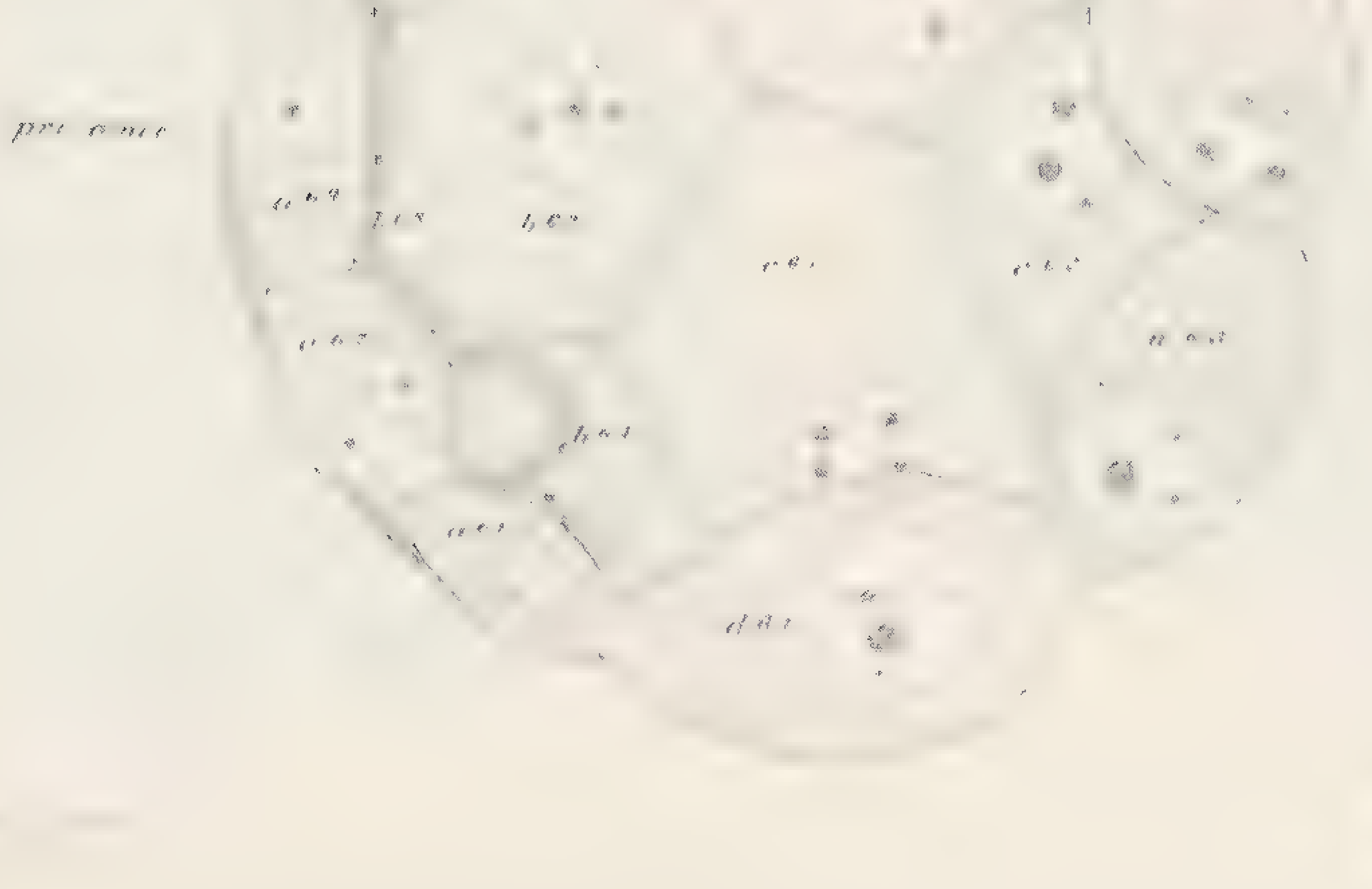


PLATE VI.

Figs. 37, 38. Forty-cell stage, composed of the following cells: —

$a^{6\cdot 8}-d^{6\cdot 8}$; $a^{6\cdot 7}-d^{6\cdot 7}$; $a^{6\cdot 6}-d^{6\cdot 6}$; $a^{6\cdot 5}-d^{6\cdot 5}$; $a^{6\cdot 4}-d^{6\cdot 4}$; $a^{6\cdot 3}-d^{6\cdot 3}$; $a^{7\cdot 4}-d^{7\cdot 4}$; $a^{7\cdot 3}-d^{7\cdot 3}$; $a^{7\cdot 2}-d^{7\cdot 2}$; $a^{7\cdot 1}-d^{7\cdot 1}$.

Fig. 37. View from the animal pole. × 490.

Fig. 38. View from the vegetative pole. Division of $d^{7\cdot 2}$ about to take place. The first mesoderm cell ($d^{7\cdot 2}=M$) with a large nucleus lies beneath its sister cell, $d^{7\cdot 1}$, only a small portion of it (shaded very dark) coming to the surface. × 490.

Figs. 39, 40. Forty-two-cell stage, composed of the following cells: —

$a^{6\cdot 8}-d^{6\cdot 8}$; $a^{6\cdot 7}-d^{6\cdot 7}$; $\frac{a^{6\cdot 6}}{a^{6\cdot 5}}$, $\frac{b^{6\cdot 6}}{b^{6\cdot 5}}$, $c^{5\cdot 3}$, $\frac{d^{6\cdot 6}}{d^{6\cdot 5}}$; $a^{7\cdot 8}-d^{7\cdot 8}$; $a^{7\cdot 7}-d^{7\cdot 7}$; $a^{6\cdot 3}-d^{6\cdot 3}$; $a^{7\cdot 4}-d^{7\cdot 4}$; $a^{7\cdot 3}-d^{7\cdot 3}$; $a^{7\cdot 2}-d^{7\cdot 2}$; $a^{7\cdot 1}-d^{7\cdot 1}$.

Fig. 39. View of the animal pole. Division of $b^{5\cdot 3}$ into $b^{6\cdot 5}$ and $b^{6\cdot 6}$. Vacuolation at animal pole. × 490.

Fig. 40. View of the vegetative pole of the same egg. × 490.

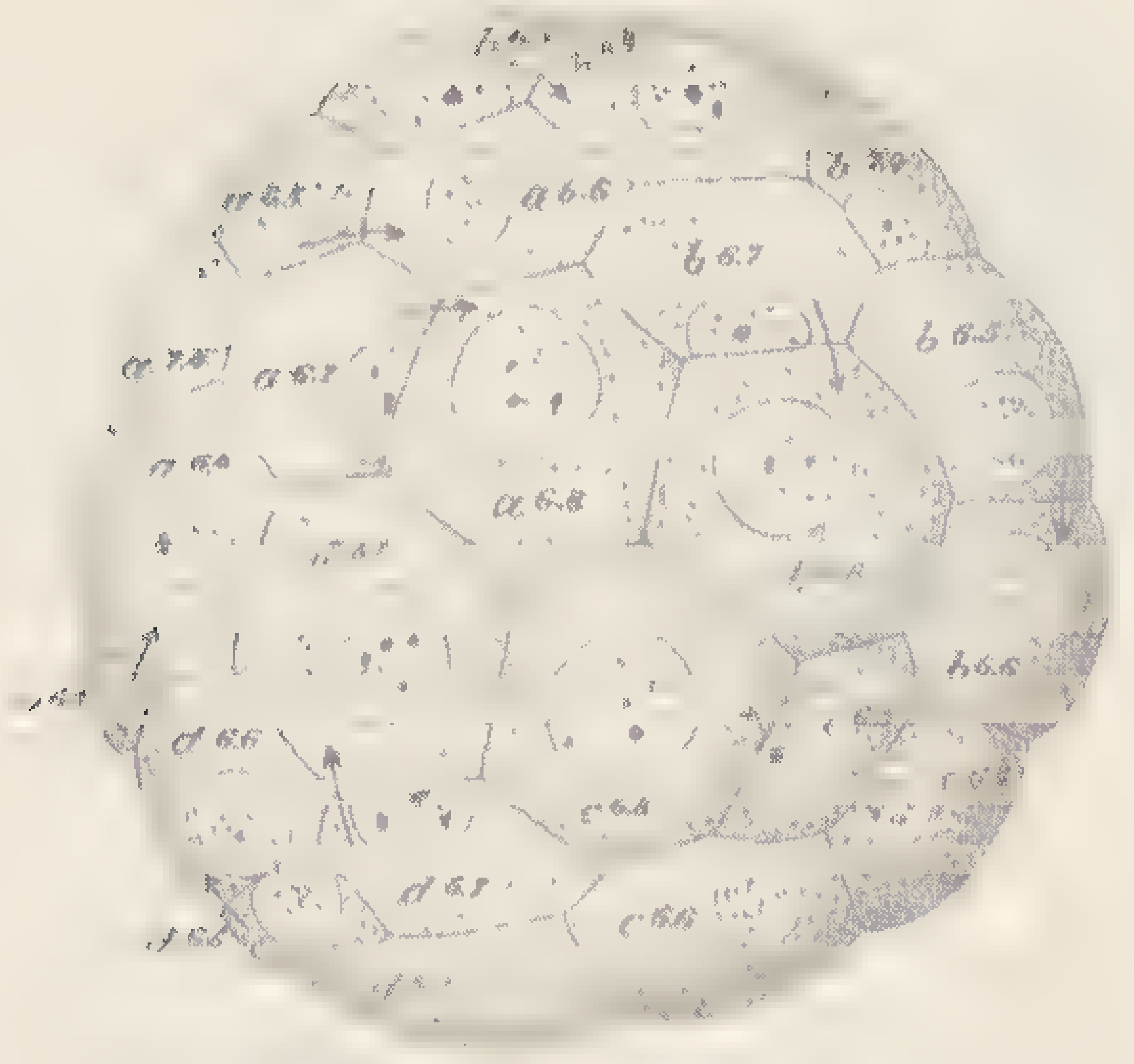
Figs. 41, 42. Forty-five-cell stage, composed of the following cells: —

$a^{6\cdot 8}-d^{6\cdot 8}$; $a^{6\cdot 7}-d^{6\cdot 7}$; $a^{6\cdot 6}-d^{6\cdot 6}$; $a^{6\cdot 5}-d^{6\cdot 5}$; $a^{7\cdot 8}-d^{7\cdot 8}$; $a^{7\cdot 7}-d^{7\cdot 7}$; $a^{6\cdot 3}$, $b^{6\cdot 3}$, $\frac{c^{7\cdot 6}}{c^{7\cdot 5}}$, $d^{6\cdot 3}$; $a^{7\cdot 4}-d^{7\cdot 4}$; $a^{7\cdot 3}-d^{7\cdot 3}$; $a^{7\cdot 2}-p^{7\cdot 2}$; $a^{7\cdot 1}-d^{7\cdot 1}$.

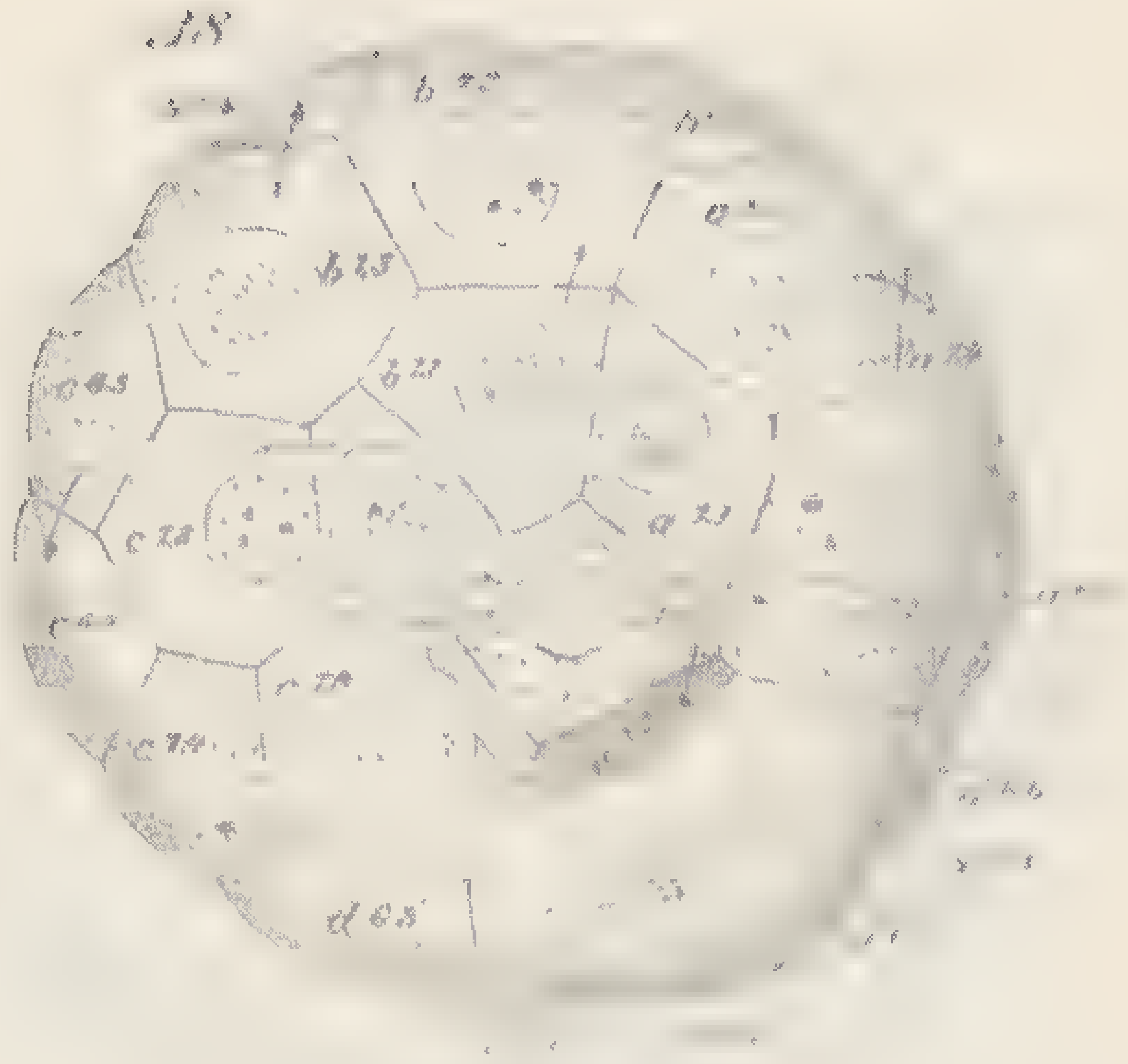
Fig 41. View of the animal pole. Recent divisions resulting in $a^{6\cdot 5}$, $a^{6\cdot 6}$, and $c^{7\cdot 5}$, $c^{7\cdot 6}$. × 490.

Fig. 42. View of the vegetative pole of the same egg. × 490.

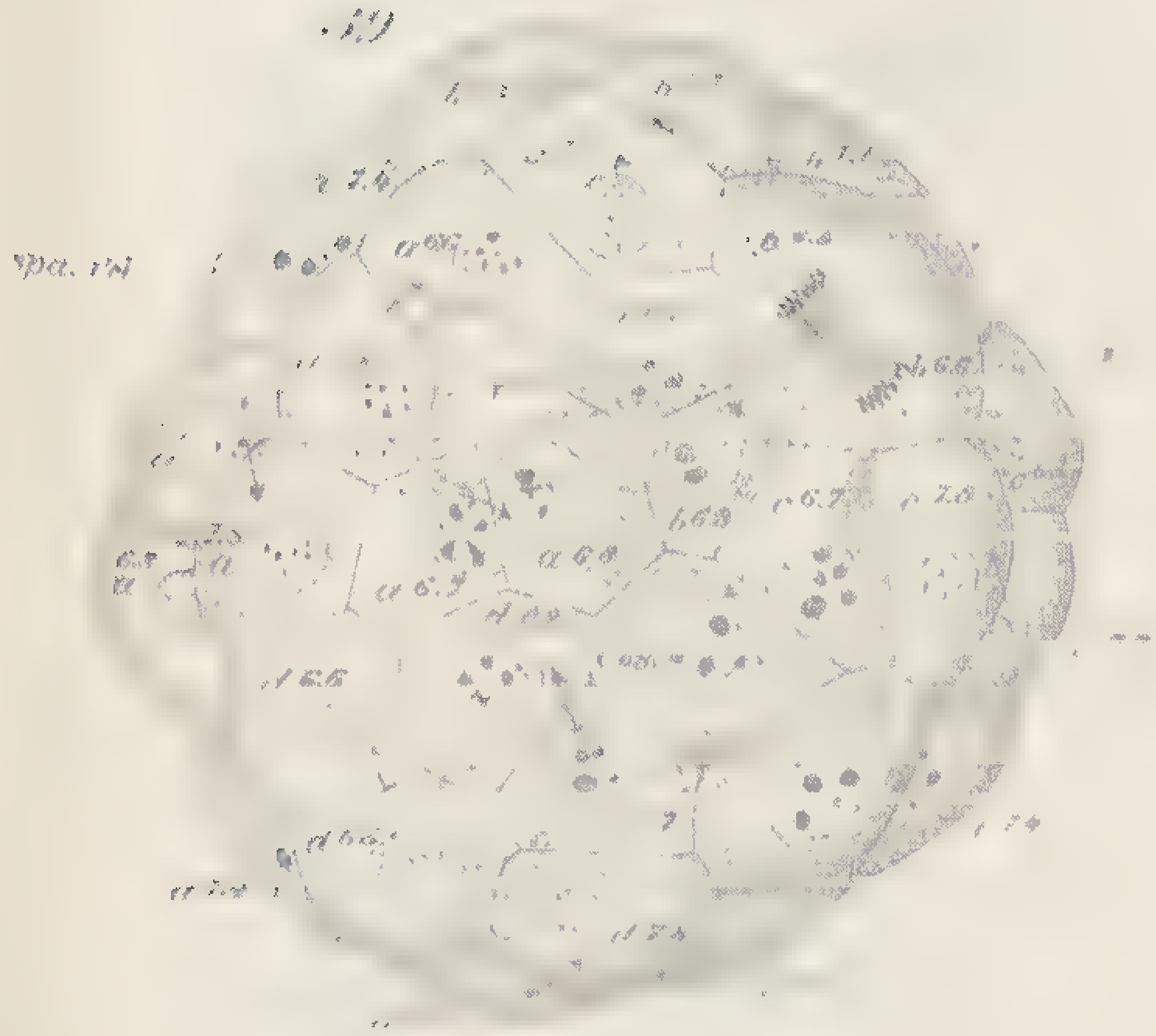
37


a 6.6
b 6.7
b 6.5
a 6.7
a 6.8
b 6.6
d 6.6
d 6.7
c 6.6

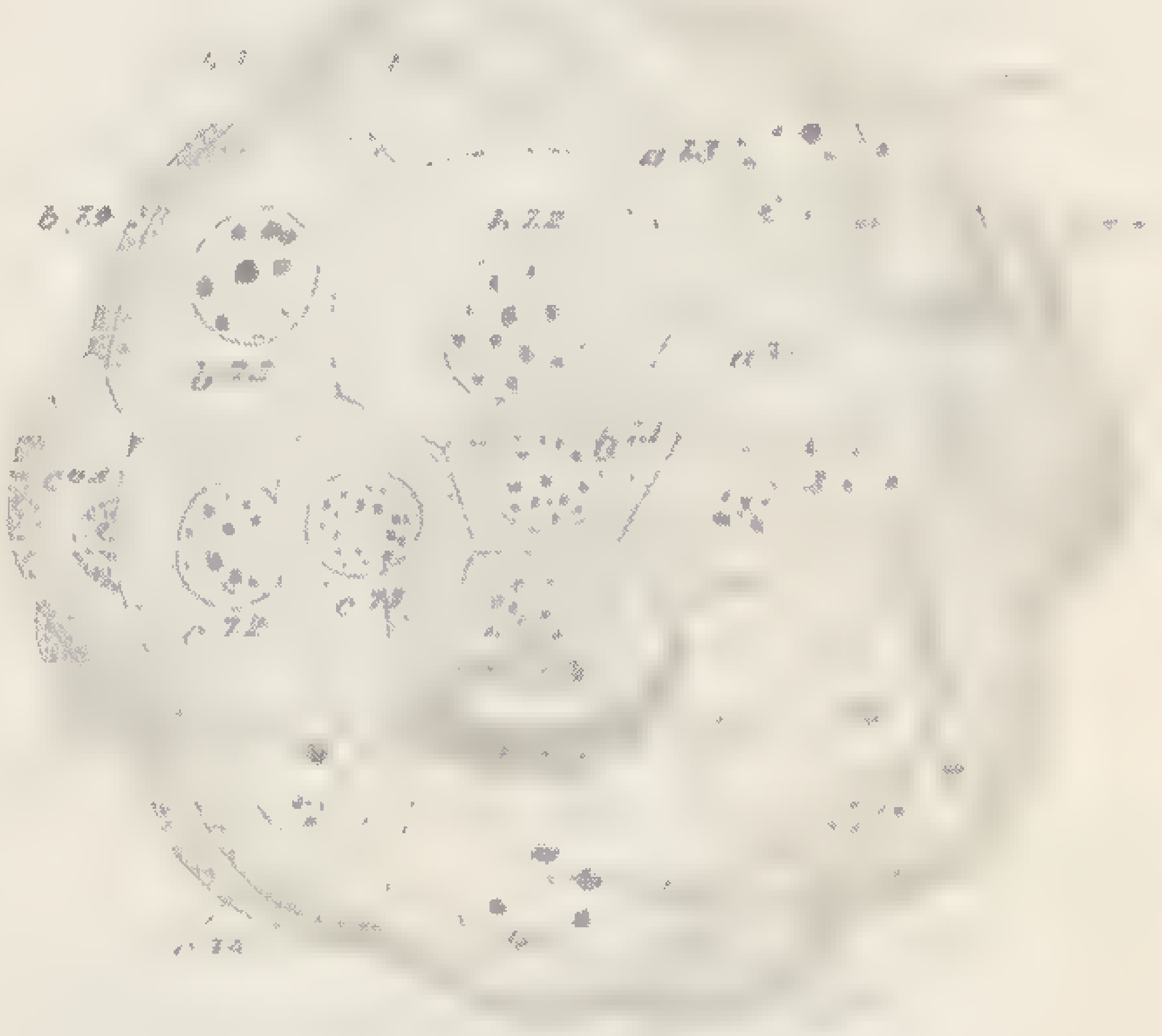
38


b 7.3
c 7.3
a 7.1
c 7.4
d 6.5

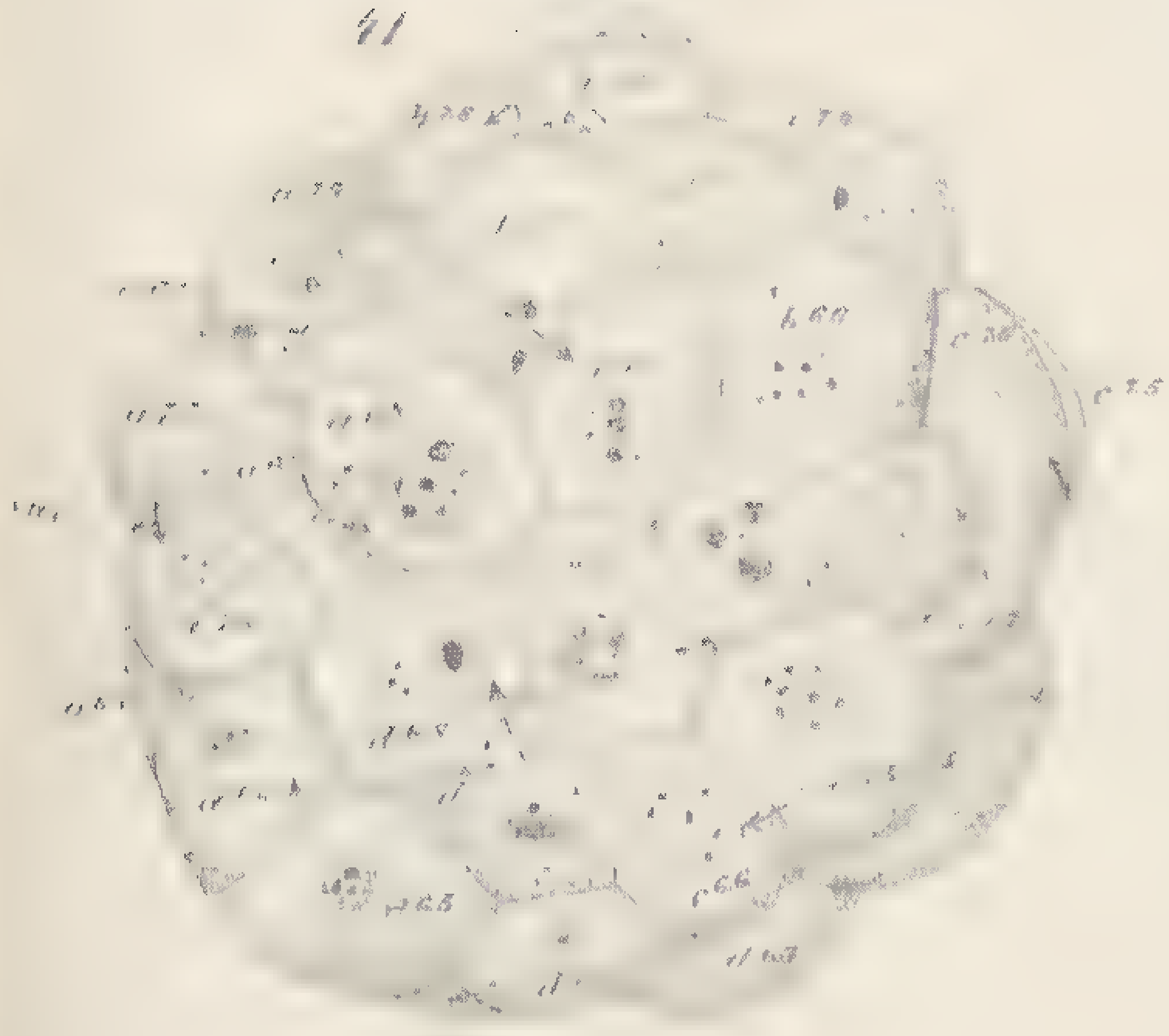
39


pa. rd
a 6.6
b 6.8
b 6.9
c 6.7
c 7.0
a 6.9
a 6.7
d 6.6
d 7.5

40


a 7.3
b 7.3
b 7.2
c 7.2

41


b 6.8
c 7.5
c 6.6
d 6.7

42

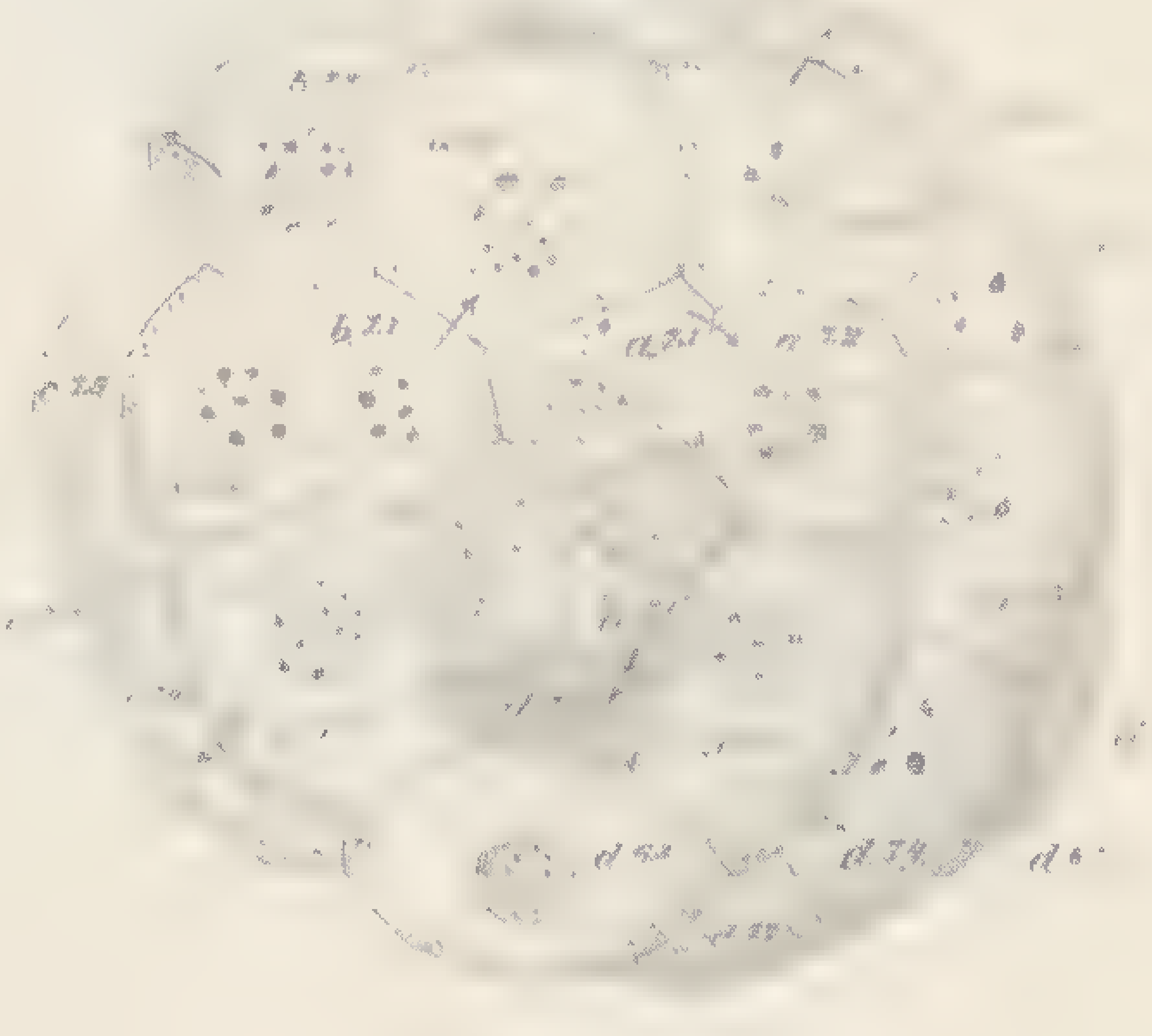

b 7.4
b 7.1
a 7.1
c 7.3
d 7.4

PLATE VII.

Fig. 43. Young gastrula, seen from the posterior end, showing the broad shallow blastopore. × 300.

Fig. 44. The same, viewed from the ventral surface. × 300.

Figs. 45–50. Sections of gastrula stage. Mesoderm cells shaded dark.

Fig. 45. View of the *posterior* face of the ninth section from the posterior end in a series of nineteen transverse sections, showing broad posterior portion of the blastopore. × 350.

Fig. 46. View of the *posterior* face of the fifteenth section in the same series, showing the deepened anterior end of the invagination. × 350.

Fig. 47. View of the *right* face (animal pole uppermost) of the eighth section in a series of sixteen sagittal sections through a blastula containing eighty cells, showing mesodermal pole cell and cleavage cavity. × 350.

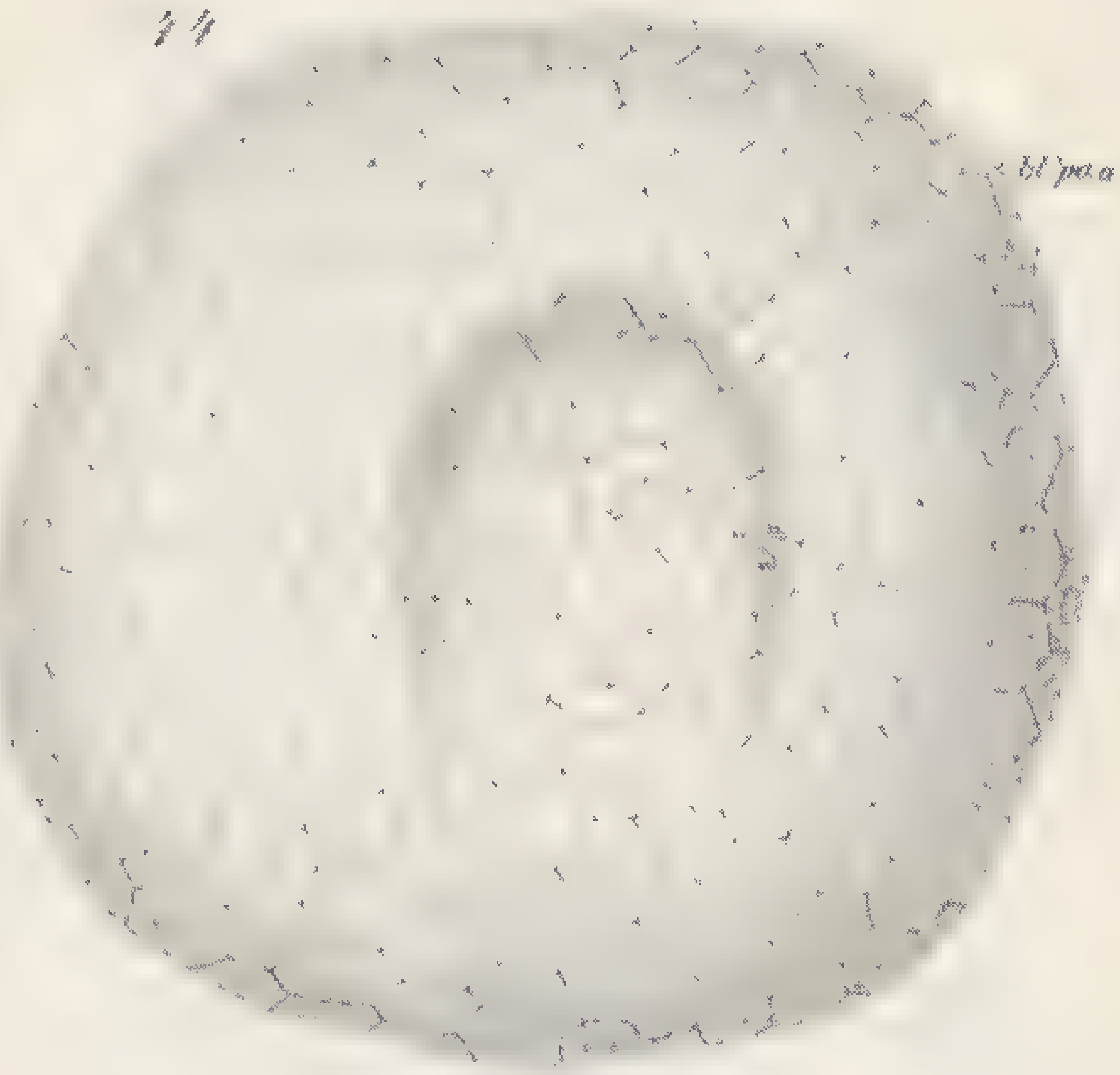
Fig. 48. View of the *left* face (animal pole above) of the eighth section of a series of twenty sagittal sections of an early gastrula, at about the stage of Figure 43, showing cleavage cavity and the mesodermal strand of the left side. × 350.

Fig. 49. View of the *left* face of the tenth section of the same series, showing at the right the mesodermal pole cell of the left side. × 350.

Fig. 50. View of the *left* face of the eleventh section of a series of seventeen sagittal sections through an embryo of about the stage of Figure 43, showing the mesodermal strand of the left side. *A.* Anterior; *P.* Posterior. × 350.

43

44



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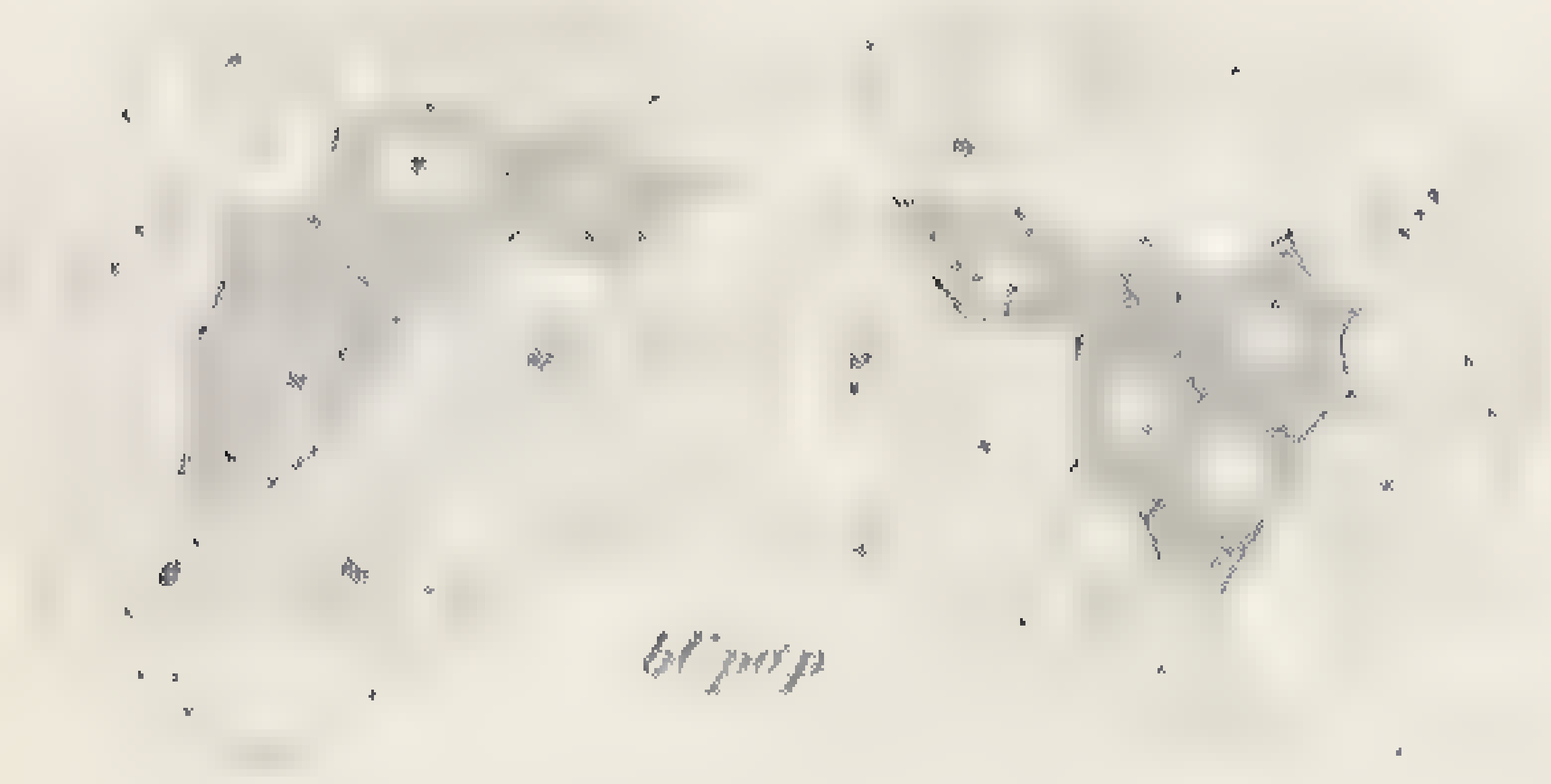

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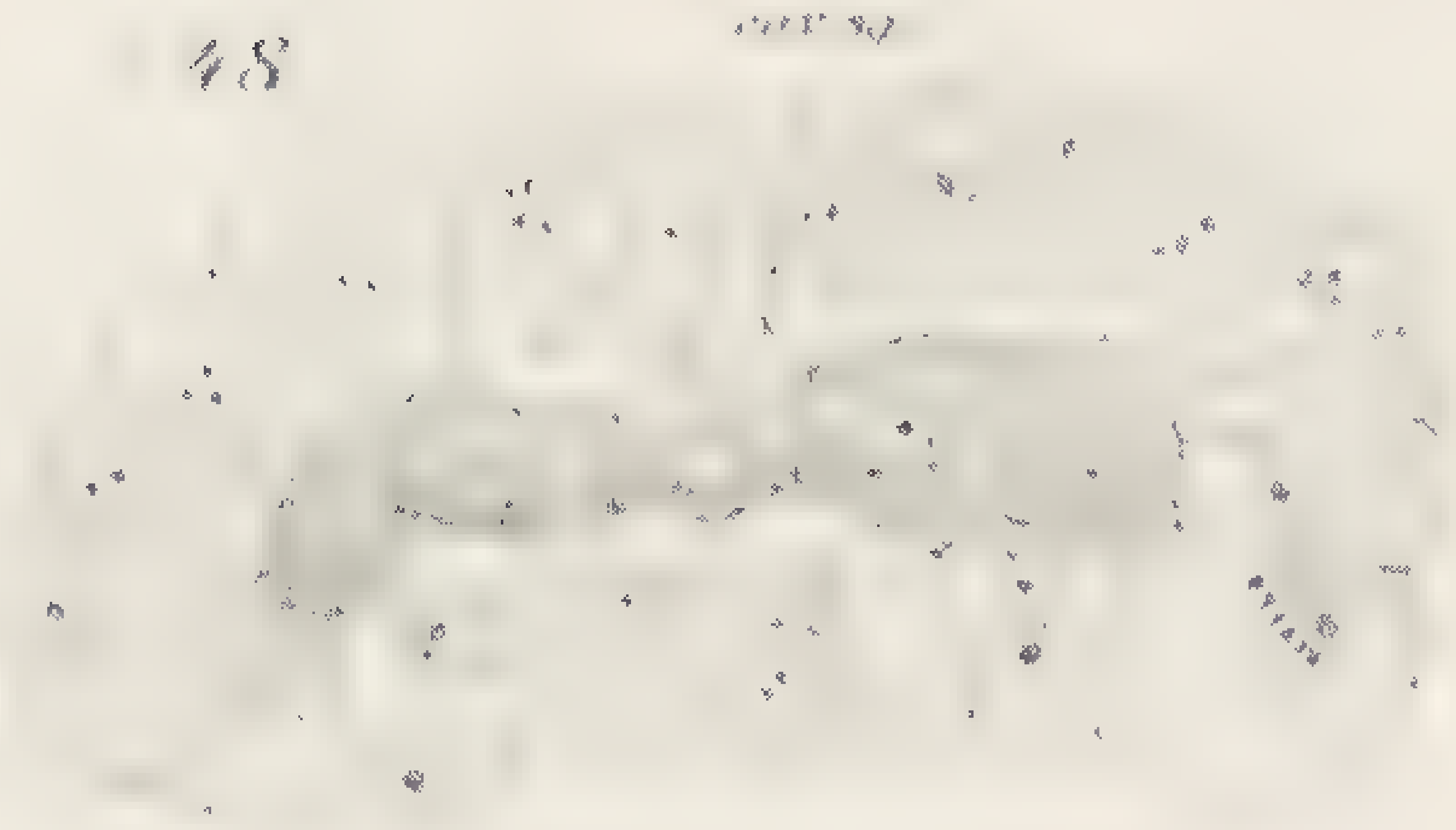

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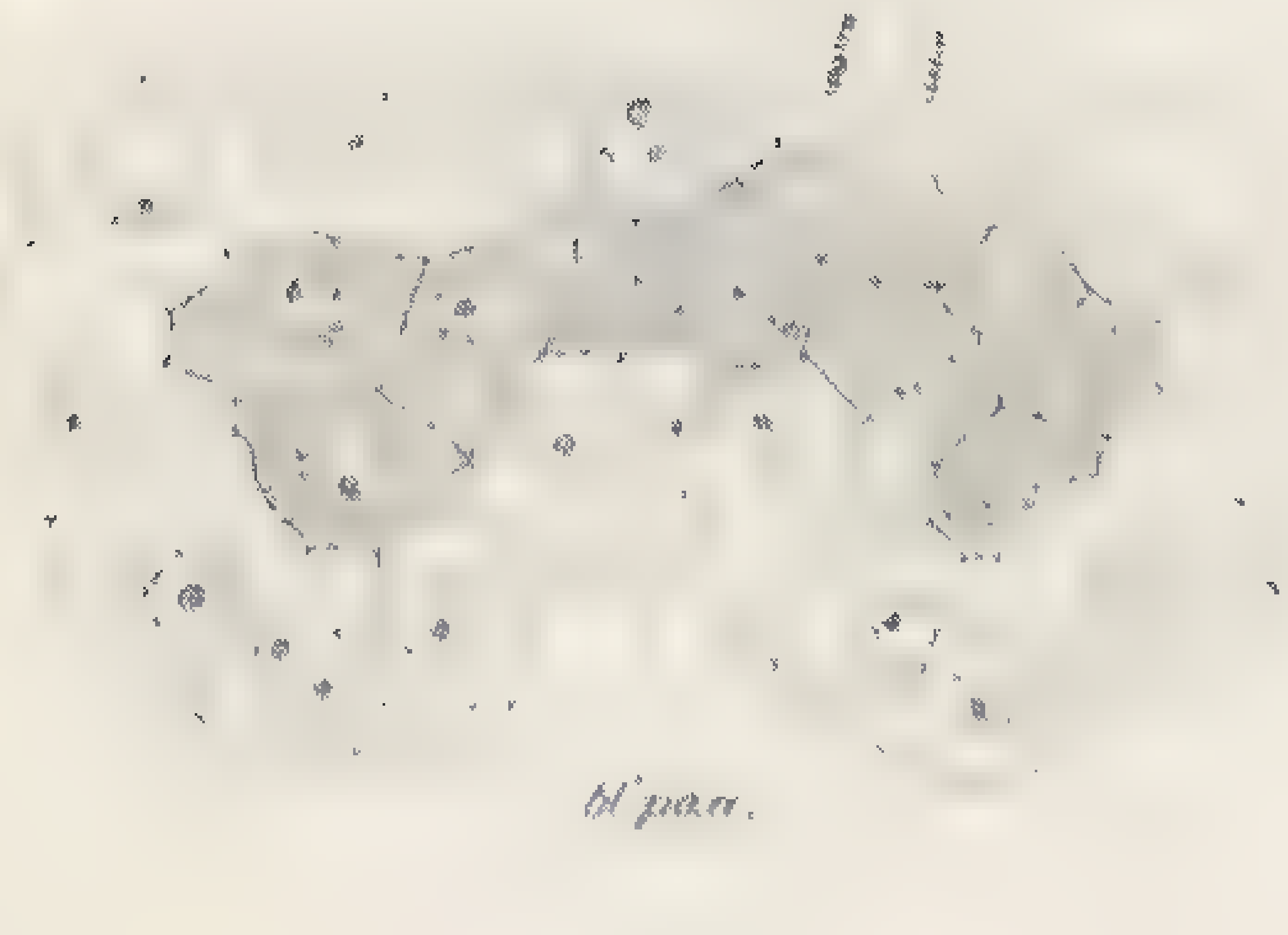

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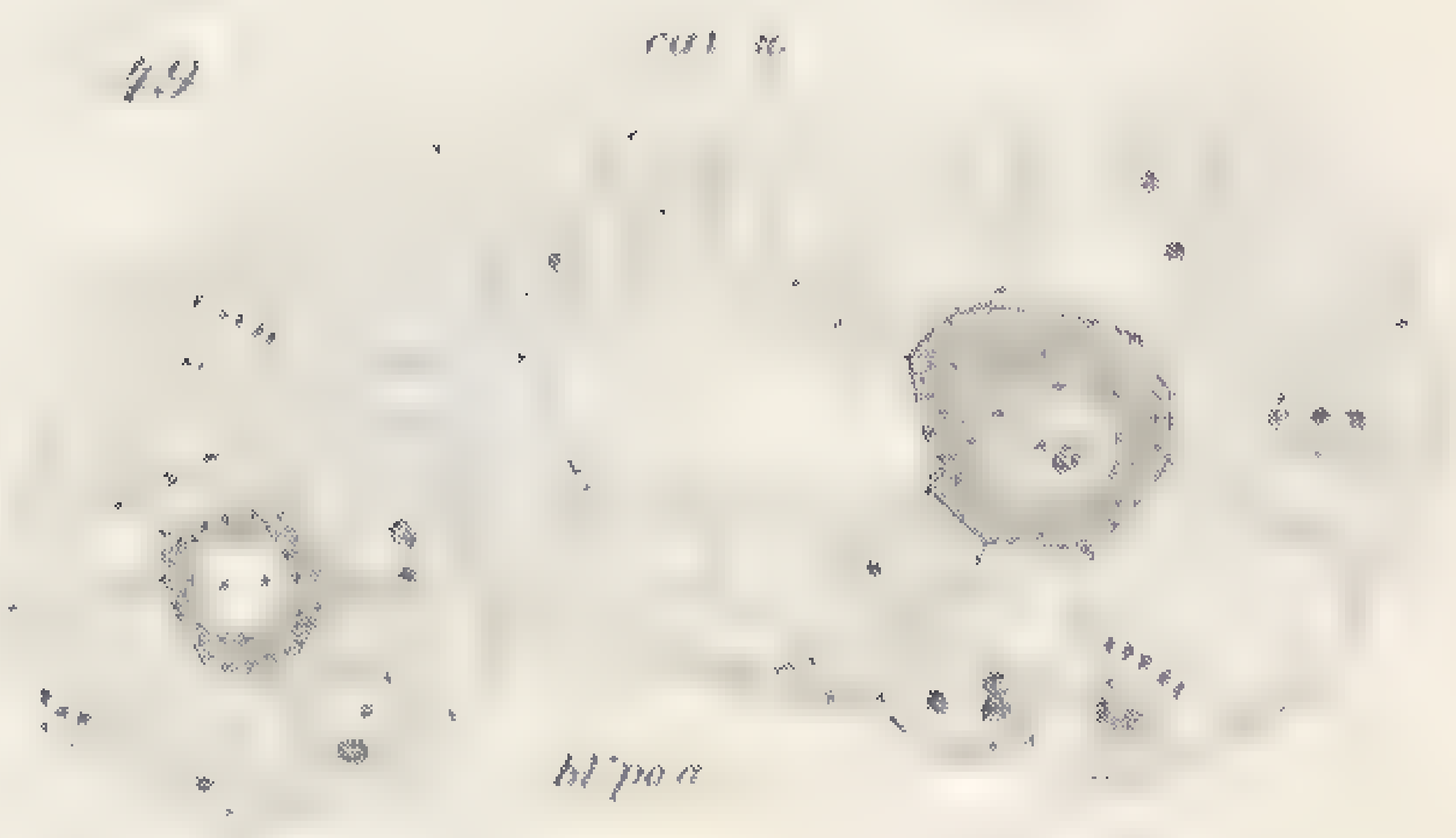


47

50

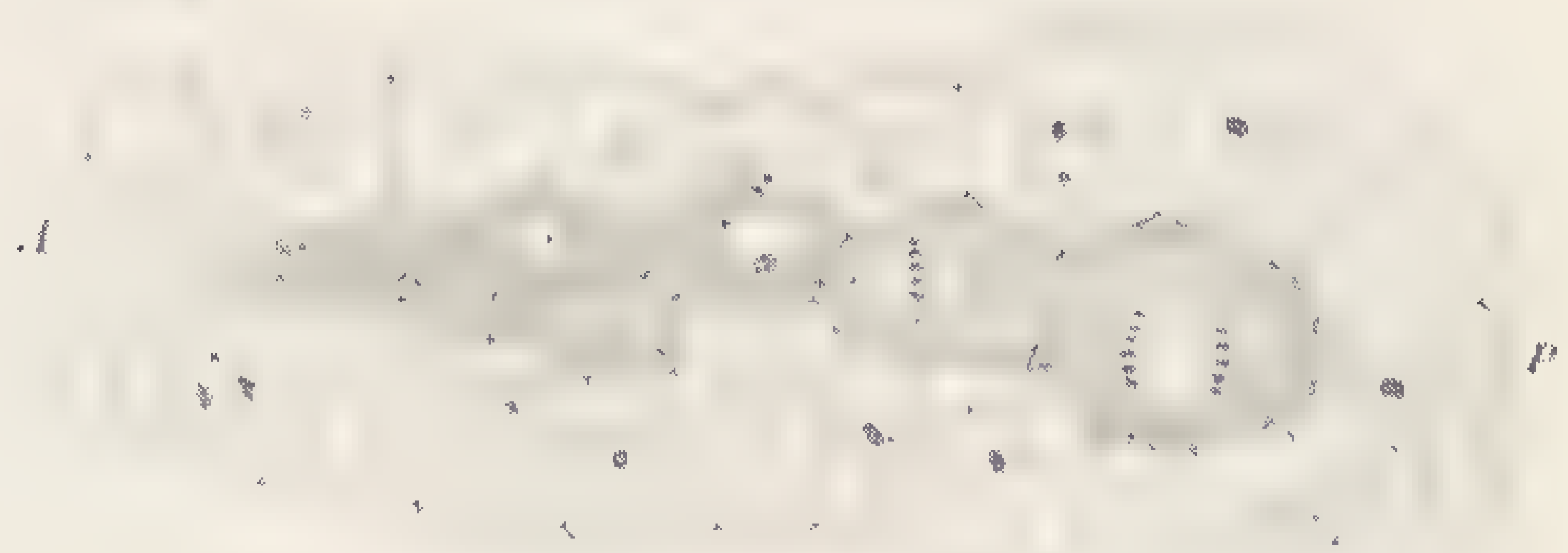


PLATE VIII.

Fig. 51. View of the ventral surface of a gastrula, showing blastopore and velar projections. × 350.

Fig. 52. View of the *anterior* face of the eleventh section from the anterior end in a series of nineteen transverse sections of an embryo of the stage of Figure 51, showing blastopore and velar projections. × 350.

Fig. 53. View of the posterior ventral surface of a gastrula somewhat more advanced than that represented in Figure 51. × 300.

Note. — A defect in the shading causes the floor of the median groove (*bl'po.*) to appear elevated into a ridge. There is no such ridge.

Fig. 54. View of the *left* face of the seventeenth section in a series of obliquely sagittal sections of an embryo of the stage of Figure 53, showing cleavage cavity at the posterior end of embryo. × 350.

Fig. 55. View of the ventral surface of an embryo more advanced than that seen in Figure 53, showing development of the cephalic vesicle and the foot. × 490.

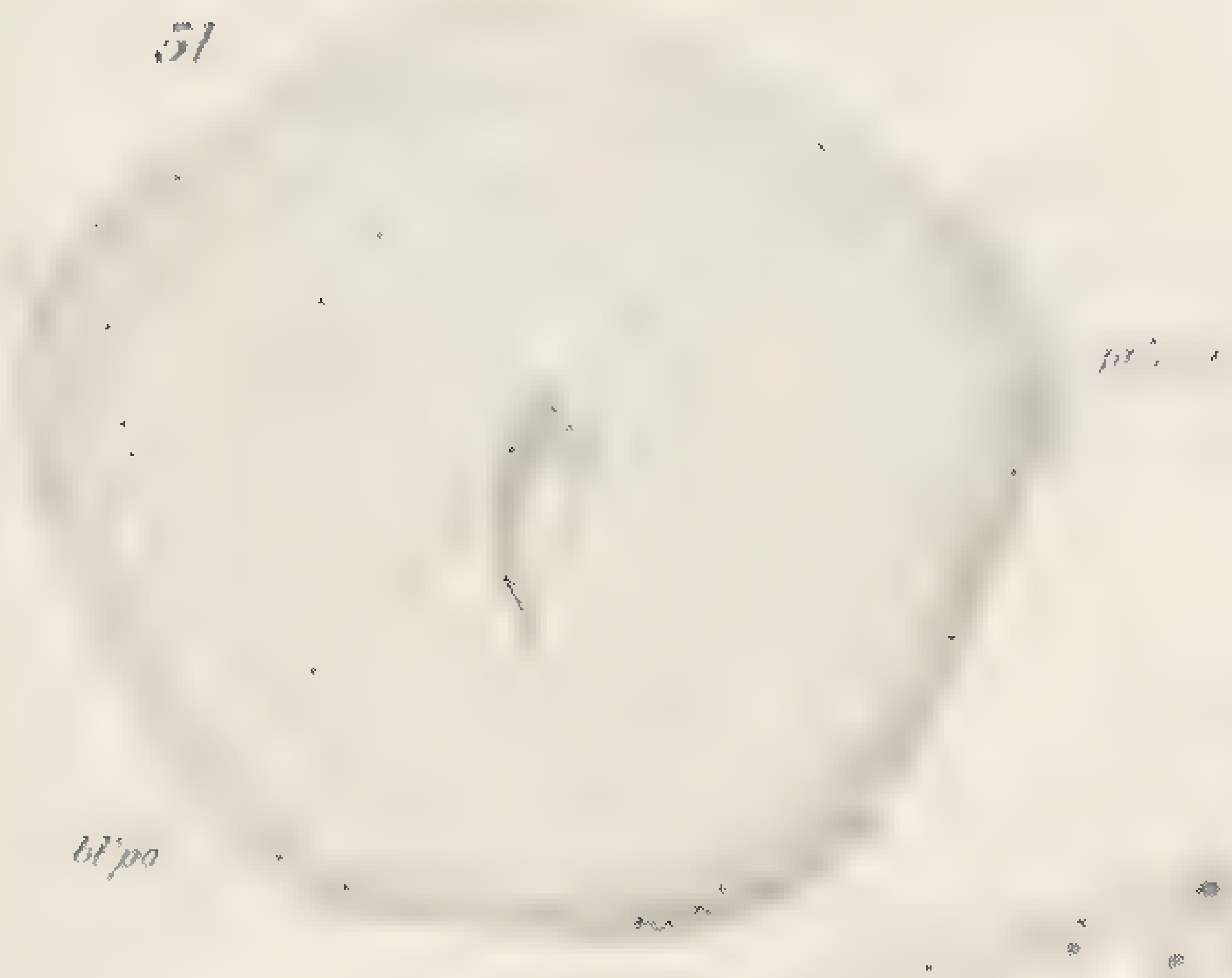
Fig. 56. View of the *right* face of the tenth section of a series of twenty-three obliquely sagittal sections of an embryo with blastopore in the posterior position. × 350.

Fig. 57. View of the *anterior* face of the eighth section, from the anterior end, of a series of sixteen transverse sections, showing archenteron, the velar ridge and enlarged cells (*cnch.*) in the region of the future shell gland. × 300.

52

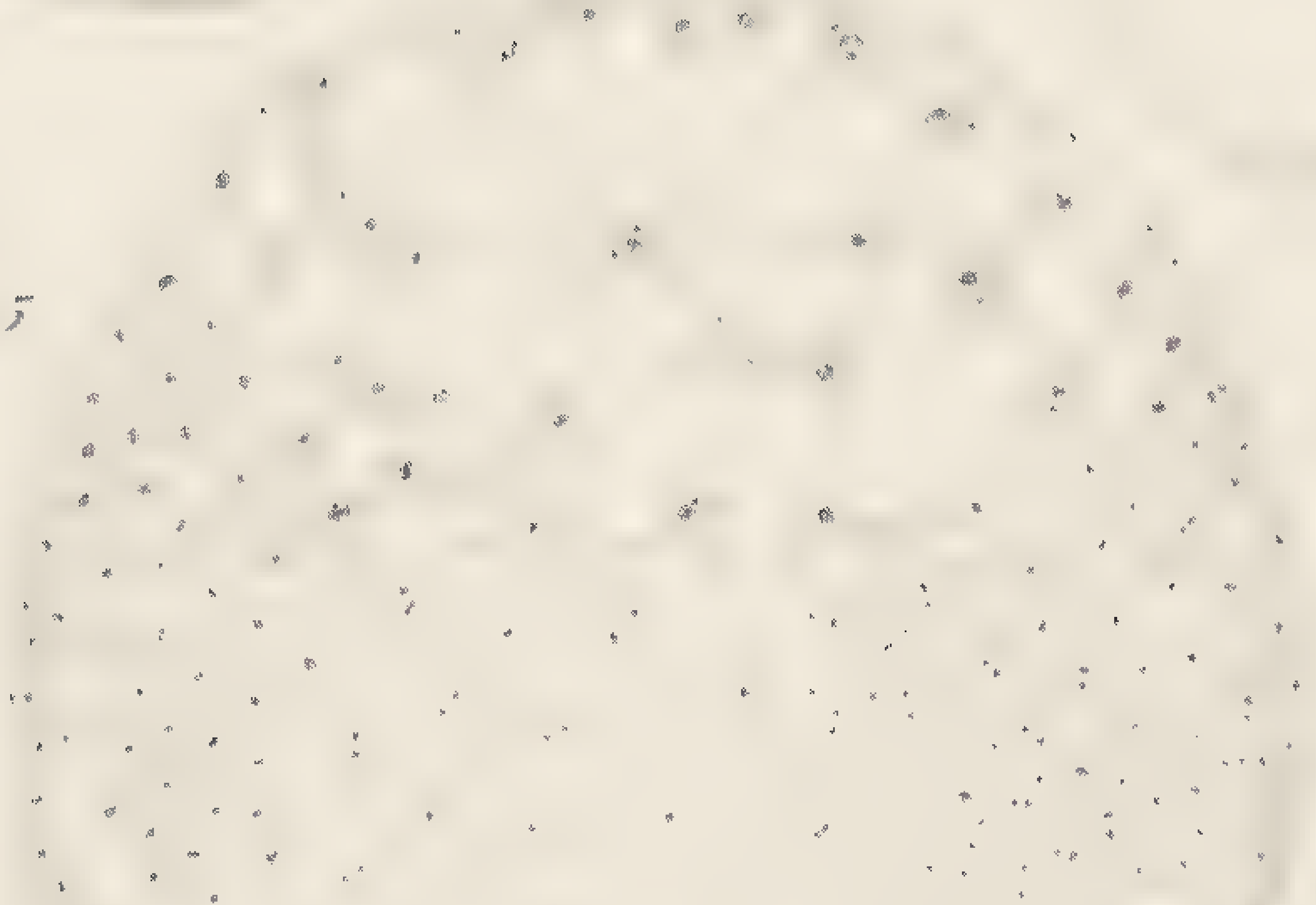
54

51



53

55



57

Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
VOL. XXVII. No. 3.

REPORTS ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

XVII.

BIRDS FROM COCOS AND MALPELO ISLANDS, WITH NOTES ON PETRELS OBTAINED AT SEA.

BY C. H. TOWNSEND.

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WITH TWO COLORED PLATES.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
JULY, 1895.

No. 3. — *Reports on the Dredging Operations off the West Coast of Central America to the Galapagos, to the West Coast of Mexico, and in the Gulf of California, in charge of* Alexander Agassiz, *carried on by the U. S. Fish Commission Steamer "Albatross," during* 1891, Lieut. Commander Z. L. Tanner, U. S. N., Commanding.

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XVII.

Birds from Cocos and Malpelo Islands, with Notes on Petrels obtained at Sea. By C. H. Townsend.

Previous to the time the "Albatross" called at Cocos Island, on February 28, 1891, nothing was known of its birds further than that it was the home of a peculiar cuckoo (*Coccyzus ferrugineus*, Gould), a single specimen of which was obtained during the voyage of H. M. S. "Sulphur," about the year 1840.

Although the collection made by the "Albatross" is a small one, but three additional genera (and species) of land birds having been found, it is interesting as showing ornithological relationship between Cocos Island and the Galapagos Archipelago.

Cocos Island is about 275 miles distant from Costa Rica, in latitude 5° 32′ 57″ N., longitude 87° 2′ 10″ W. It occupies a position nearly midway between the mainland and the islands of the Galapagos group, and with the exception of Malpelo Island, an inaccessible barren rock off the Gulf of Panama, is the only connecting point of land. Like the Galapagos Islands, it is of volcanic origin, and has received its peculiar animal and vegetable forms from the mainland. The American origin of the forms of life upon the Galapagos Islands was demonstrated by Darwin, who made researches there more than half a century ago. It appears from a study of the birds alone, that Cocos Island is similarly a satellite of America, with the added interest of being a stepping-stone to the group of islands beyond it, some of whose ornithological features it bears.

Darwin, the first to study the birds of the Galapagos Islands, described remarkable variations among them, even those inhabiting the same island, that made it difficult to separate them specifically. New forms brought to light by recent explorations, particularly those of the "Albatross," have only served as links to connect the species still more intimately, so that upon the Galapagos Islands there exists the most remarkable grading together of species known to ornithology. This is especially noticeable in the group of finches, in distinguishing which arbitrary measurements are employed, some of the smaller forms closely approaching *Certhidea*, a genus of the *Cærebidæ*. Into the gap between these (*Cactornis* and *Certhidea*) *Cocornis* from Cocos Island seems to fit. The relationship of the Cocos Island flycatcher *Nesotriccus* is equally close to *Eribates* inhabiting the Galapagos. In view of these facts, it is to be regretted that our limited stay at Cocos Island did not permit of a more thorough search for birds, as it is possible that other species exist in the elevated central part of the island which we were unable to reach.

The island is about four miles long by three wide, its central part having an elevation of about 1,700 feet. It is everywhere covered with the densest forest. Cocoanut trees are found upon the higher slopes, and tree ferns abound in the ravines. No tropical forest could be more dense and tangled. The rainfall is doubtless great, as each ravine contains a dashing stream. It is a garden spot in comparison with the arid Galapagos Islands.

I am indebted to the kindness of Mr. Ridgway, Curator of Birds in the National Museum, for much valuable information on the ornithology of the islands of this region.

COCOS ISLAND.

Dendroica aureola, Gould.

Sylvicola aureola, Gould, Zoöl. Voy. Beagle, Part III. p. 86.
Dendrœca aureola, Salv., Trans. Zoöl. Soc. Lond , Vol. IX. Part IX. p. 473.

The two specimens of this warbler secured are not distinguishable from the species (*D. aureola*) inhabiting the Galapagos. Only one other was seen. The species is more closely related to *D. petechia* from Jamaica than the species of the mainland.

Cocornis,[1] gen. nov.

Intermediate between *Cactornis* and *Certhidea* of the Galapagos Islands, but distinguished from both in having a decidedly curved bill. The commissure is without the pronounced angle of the former and the gentle curve of the latter. It is nearest *Cactornis*, which it resembles in feet, coloration, and size, differing in these respects from *Certhidea*, which it resembles more in the slender character of its bill.

Type Cocornis Agassizi, sp. nov.

Specific characters similar to *Cactornis scandens*, but with bill more slender and curved, and less rounded, the culmen having more of the character of a ridge.

Hab. Cocos Island.

Adult male (Type No. 131680, Cocos Isl., Feb. 28, 1891, C. H. T.). Uniform sooty black, except on under tail coverts, which are tipped with buff. Bill dark brown, lower mandible lighter; legs and feet brownish black. Length (skin), 4.85 inches; wing, 2.60; tail, 1.80; culmen, .56; gonys, .35; bill from rictus, .60; tarsus, .80; middle toe, .50.

Immature male? (No. 131682). Sooty black, washed with olive-buff, especially below and on under tail coverts. Length (skin), 4.70; wing, 2.60; tail, 1.75; culmen, .56; gonys, .35; bill from rictus, .60; tarsus, .80; middle toe, .50.

Adult female? (No. 131690). Above sooty black, but with the feathers extensively edged and tipped with tawny olive, especially on upper tail coverts, where the black is almost entirely obscured. Edging fainter anteriorly, leaving crown quite dark. Middle and greater wing coverts edged and tipped with russet, tail russet-tipped. Below olive-buff, with the black appearing as a central streak in each feather, except on belly and under tail coverts, which are almost entirely olive-buff. The coloration of the upper parts blends gradually on sides, into that of the lower parts. Quills and tail narrowly edged with russet. Bill pale with dark tip. Legs and feet black. Length (skin), 4.50 in.; wing, 2.50; tail, 1.65; culmen, .50; gonys, .30; bill from rictus, .55; tarsus, .80; middle toe, .50.

In a series of eleven specimens of this bird, six males are sooty black, two of them entirely so (including the type specimen). Two have the bill entirely black. The other dark males have the bill dark brown. Five specimens, two females and three young males, resemble the female described above, and have the bill pale. The young males are somewhat darker than the females, while the full series of males exhibits a regular gradation from the light color of the female to the very dark color of the adult male. This is the only land species that seems to be really common. It is finch-like in its habits, always actively flitting from branch to branch.

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[1] *Cocos* = the cocoa palm from which the island derives its name; ὄρνις = bird.

This remarkable species is named for Professor Alexander Agassiz, who was in charge of the work of the "Albatross" at the time Cocos Island was visited.

Nesotriccus, gen. nov.[1]

Allied to *Eribates* of the Galapagos Islands, but with bill relatively longer and more flattened. Culmen separating the nostrils as a prominent ridge. Gonys less than half the length of lower mandible, terminating in advance of nostrils. Tail relatively shorter.

TYPE Nesotriccus Ridgwayi, sp. nov.

Specific characters. Distinguished from the allied *Eribates magnirostris* in having no trace of rufous on inner webs of tail feathers, and no ashiness of throat and breast. It is also smaller, with nostrils separated by a sharp ridge.

Hab. Cocos Island.

Adult male (Type No. 131691, Cocos Isl., Feb. 28, 1891, C. H. T.). Above olive, brightening to olive-buff on rump; tips of middle and greater wing coverts creamy buff; wings and tail dusky, with narrow olive-buff edgings. Below olive, suffused with yellow, brighter on belly and under wing and tail coverts, darker on breast and sides of head and neck: throat pale buff. Bill dark brown, with posterior half of lower mandible pale yellow. Legs and feet dark brown. Length (skin), 5.25 in.; wing, 2.40; tail, 2.20; culmen, .55; gonys, .35; bill from rictus, .80; depth at base, .18; tarsus, .80; middle toe, .45.

Only one specimen of this bird was obtained, and to the best of my recollection only two or three others seen. They were observed among the tree-ferns in a deep ravine at Chatham Bay. The species is named for Mr. Robert Ridgway, Curator of Birds in the U. S. National Museum.

Coccyzus ferrugineus, GOULD. (*Nesococcyx*, Cab.)

Coccyzus ferrugineus, Gould, Proc. Zoöl. Soc., 1843, p. 104. Zoöl. Voy. Sulph., Birds, I. p. 46.

Only two specimens of this bird were obtained, and not more than three or four others seen. As in the case of the warbler (*Dendroica*) its relationships are with species inhabiting the West Indies, rather than with the forms of the mainland. The genus was not known to the Galapagos Islands until the voyage of the "Albatross," in 1888, when two specimens of *Coccyzus melanocoryphus* Vieill., a mainland form, were secured on Chatham and Charles Islands.

---

[1] νῆσος = island; *Triccus* = a genus of tyrant flycatchers.

Anous stolidus, LINN.

Sterna stolida, Linn., Syst. Nat., Vol. I. p. 227.
Anous stolidus, Ridgw., Proc. U. S. N. M., Vol. XII. p. 116.

Abundant; four specimens collected. This species was noticed as most numerous, flying among the branches of the trees in the forest. The specimens, although resembling *A. galapagensis*, Sharpe, are apparently referable to *A. stolidus.*

Sula, sp.

Abundant, not collected.

MALPELO ISLAND.[1]

Creagrus furcatus (NÉBOUX).

Larus furcatus (Néboux), Prev. et des Murs, Voy. Venus, V. Ois., p. 277.
Creagrus furcatus, Salv., Trans. Zoöl. Soc. Lond., Vol. IX. Part IX. p. 506.
Creagrus furcatus, Ridgw., Proc. U. S. N. M., Vol. XII. p. 117.

I shot four specimens of this rare gull during our short stop at Malpelo Island on March 5th, and saw several others upon the cliffs. The species is doubtless abundant there, as sea birds of several species swarm about the inaccessible summit of the island.

But three specimens of this bird were known prior to the voyage of the "Albatross" to the Galapagos Islands in 1888, when I procured two specimens at Dalrymple Rock, Chatham Island. Malpelo Island is a new locality for *Creagrus furcatus*, the other examples known having all come from the Galapagos, with the exception of one from Peru, and the original specimen procured during the voyage of the "Venus," 1836–39, attributed, doubtless erroneously, to Monterey, California. Unless contained in the Baur and Adams collection, only nine specimens are known at the present time, although the bird was discovered more than fifty years ago.

PETRELS OBTAINED AT SEA.

Oceanodroma cryptoleucuera, RIDGW.

Three specimens obtained off Wenman Island, Galapagos, April 4. They were attracted on board by the electric lights used on deck while dredging at night.

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[1] Malpelo Island is a volcanic rock in Lat. 3° 59′ 7″ N.; Lon. 81° 34′ 27″ W. It is less than a mile in greatest length, with a height of over 800 feet. It is inaccessible and without vegetation other than a small patch of bushes.

Oceanodroma melania, Bonap.

This species from the west coast of Mexico has been described by Ridgway as *O. Townsendi* in the Proceedings of the National Museum, Vol. XVI. p. 687, but is probably referable to *O. melania.*

Nine specimens. I obtained the first on March 28, 1889, off Guaymas, the others in 1891; one off Acapulco, April 12th, and the others off Guaymas, April 21st.

Halocyptena microsoma, Coues.

Halocyptena microsoma, Coues, Proc. Phila. Acad., 1864, p. 78.

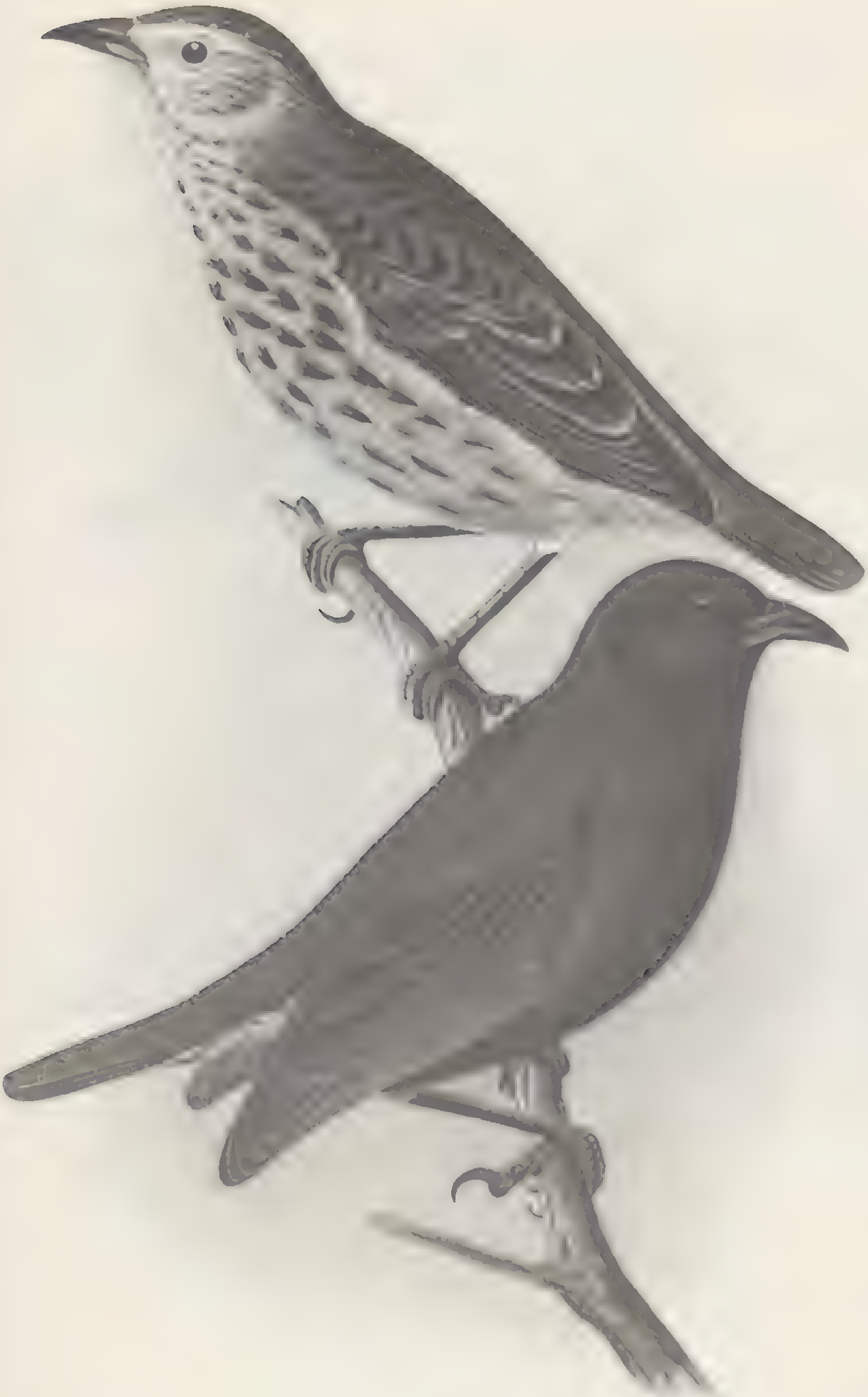
A single individual obtained while the "Albatross" was dredging off Acapulco, April 12th. This is the third specimen known. I procured the second in 1888, off Panama. The original was taken in 1861, off Cape St. Lucas.

Procellaria tethys, Bonap.

Four specimens: two off Chatham Island, Galapagos, March 28th, and two on March 24th, 400 miles east of the Galapagos.

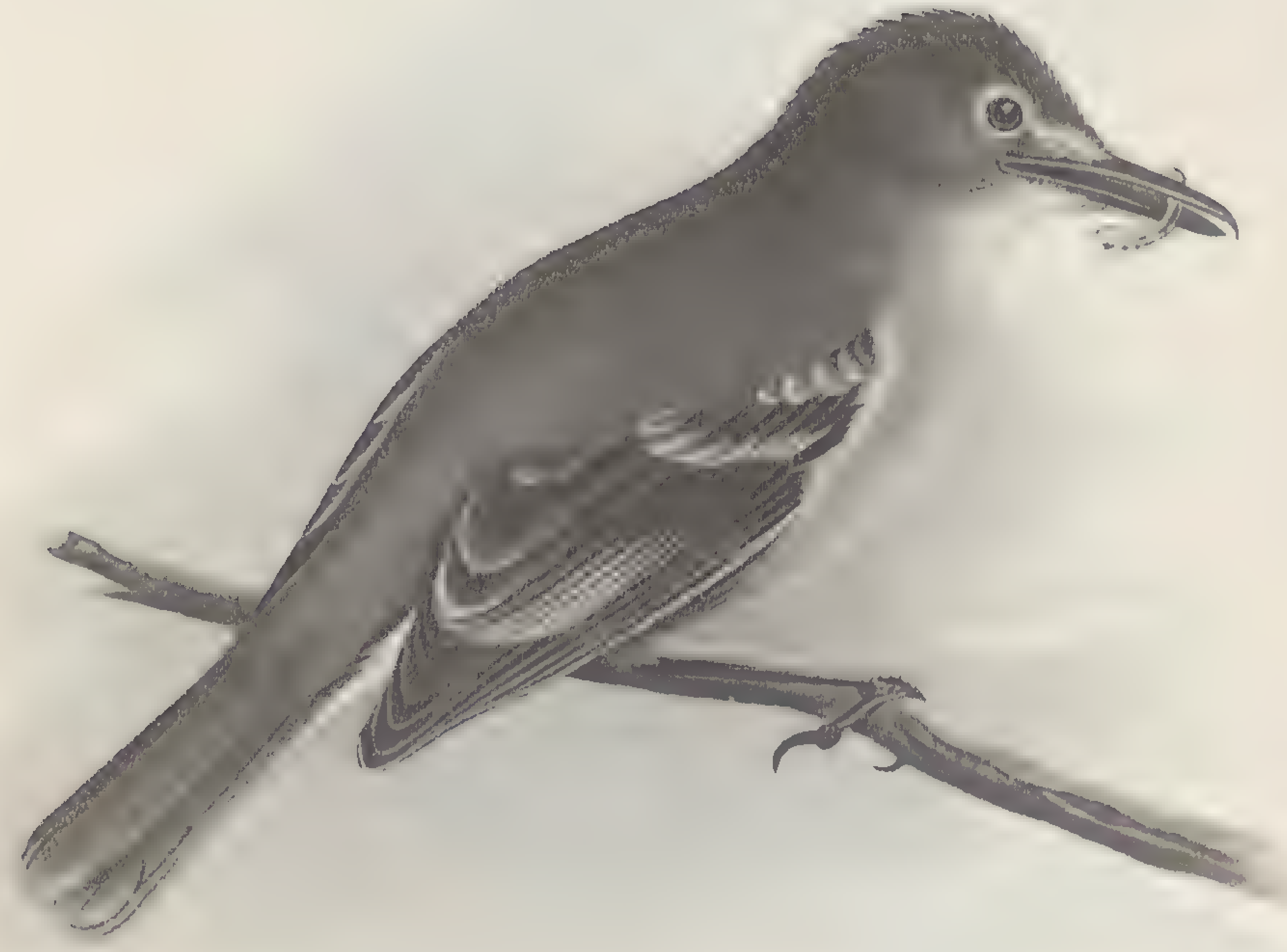
Puffinus tenebrosus, Pelz.

Three specimens: one off Chatham Island, March 28th, the others off Wenman Island, April 4th.



COCORNIS AGASSIZI, Townsend

Adult Male and Female



NESOTRICCUS RIDGWAYI, Townsend

Adult Male

Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
VOL. XXVII. No. 4.

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XVIII.

DIE COMATULIDEN.

VON C. HARTLAUB.

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WITH FOUR PLATES.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
AUGUST, 1895.

No. 4. — *Reports on the Dredging Operations off the West Coast of Central America to the Galapagos, to the West Coast of Mexico, and in the Gulf of California, in charge of* ALEXANDER AGASSIZ, *carried on by the U. S. Fish Commission Steamer "Albatross," during* 1891, Lieut.-Commander Z. L. TANNER, U. S. N., Commanding.

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XVIII.

Die Comatuliden. Von C. HARTLAUB.

Die Ergebnisse der Albatross Expedition sind, wie bereits von *Agassiz*[1] mitgetheilt wurde, auf dem Gebiete der Crinoiden auffallend spärlicher Natur gewesen. Während der Dampfer von andern Echinodermen, wie vor Allem Echiniden, Asteriden und Holothurien reiches Material erbeutete, besteht der ganze Fang an Crinoiden aus nur 7 *Antedon*-Arten und dem interessanten *Calamocrinus Diomedæ*, welchen *Agassiz* schon beschrieben hat.[2] Unter den 84 Dredge-Stationen der Reise sind nur 10 zu nennen, die überhaupt Crinoiden lieferten. Von diesen fallen 6 auf die erste Fahrt, von Panama nach Cocos Island, 3 auf die zweite (3 Stationen bei den Galapagos) und eine auf die dritte Fahrt (Sta. 3424 Las Tres Marias). Ansehnliche Mengen von Exemplaren ergaben nur Sta. 3385, Golf von Panama (*Antedon tanneri* n. sp.), Sta. 3424, Las Tres Marias, und Sta. 3357, nicht weit von Cap Mariato Point, wo u. a. eine Anzahl Antedons aus der *Basicurva*-Gruppe (*Antedon agassizii* n. sp.) und der erwähnte *Calamocrinus* erlangt wurden.

Verglichen mit den Crinoiden Schätzen der *Blake*-Expedition auf der atlantischen Seite Central-Americas war dies negative Resultat auf der pacifischen gewiss sehr überraschend; allein so klein die Zahl der mitgebrachten Arten auch ist, die Albatross-Expedition hat doch unsre Kenntniss von der geographischen Verbreitung des Genus Antedon sehr bemerkenswerth bereichert. Es hat sich nämlich herausgestellt, dass eine Artengruppe dieser Gattung, die man nach den bisher bekannten Vertretern als vorwiegend arctisch und nahezu antarctisch ansah, dies keineswegs ist. Die Eschrichti Gruppe, als deren südlichster Verbreitungspunct auf der nördl. Halbkugel der 43. Grad nördl. Br. angesehen wurde (*Antedon eschrichti bei Halifax*) und welche auf der südlichen Hemisphære die Magelhan Strasse und die Heard Islands als Fundorte hat, ist nicht minder in den Tropen zu Hause. An Individuenzahl nimmt sie in der Albatross-Sammlung sogar bei Weitem die erste Stelle ein, leider auch bezüglich der Schwierigkeit, welche mir die systematische Beurtheilung der beiden Formen gemacht hat, von denen ich nachstehend mit allem bei Crinoidenarten nöthigen Vorbehalte eine als neu beschrieben werde. Der Systematiker für Comatuliden ist ja insofern schlimm daran, als er für die mühsame Begründung neuer Arten selten durch das glückliche Gefühl voller Sicherheit belohnt wird.

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[1] *Agassiz Al.*, General Sketch of the Expedition of the "Albatross" from February to May, 1891, in: Bull. Mus. Comp. Zoöl. Harvard College. Vol. XXIII. No. 1.

[2] *Agassiz Al.*, *Calamocrinus diomedæ* in: Mem. Mus. Comp. Zoöl. Harv. Coll. Vol. XVII. No. 2.

I. ARTEN MIT AMBULACRALER TÄFELUNG.

BASICURVA GRUPPE Carp. Chall. Rep. XXVI. p. 99.

10-*armige Antedon Arten, deren Radialia und untere Armglieder abgeplattete Seiten haben, und deren Pinnula-Ambulacra meistens getäfelt sind; die zwei äusseren Radialia sind gelenkig verbunden.*

Uebersicht und Verbreitung ihrer Arten: —

Gesammtzahl der Arten, 21.

Geringste Tiefe: 49 Faden, Arapura See. — (*Antedon denticulata* Carp.)

Grösste Tiefe: — 1600 Faden, Südsee. — (*Antedon bispinosa* Carp.)

In Tiefen unter und bis zu 500 *Faden*, 13 Arten.

49 Faden,	Arapura See.		*Antedon denticulata* Carp.
88–262	"	Carribean Sea Str. of Florida.	" *duplex* Carp. M. S.
140	"	Ki Islands.	" *flexilis* Carp. " *longicirra* Carp. " *parvipinna* Carp. " *pusilla* Carp.

270	Faden,	Str. of Florida.	*Antedon*	*brevipinna* Pourt.
345	"	off Japan.	"	*latipinna* Carp.
375	"	Pacific, off Pangloa Isl.	"	*tuberosa* Carp.
420–550	"	S. Atl. off Tristan da Cunha, Ascension.	"	*multispina* Carp.
500	"	Meangis Isl.	"	*aculeata* Carp.
			"	*gracilis* Carp.
			"	*valida* Carp.

In Tiefen von über 500–1000 *Faden*, 9 Arten.

420–550	Faden,	S. Atl. Tristan da Cunha, Ascension	*Antedon*	*multispina* Carp.
630	"	Kermadecs.	"	*incerta* Carp.
			"	*echinata* Carp.
			"	*basicurva* Carp.
630–1350	"	" und Fiji.	"	*breviradia* Carp.
610–630	"	"	"	*incisa* Carp.
740	"	off Portugal.	"	*lusitanica* Carp.
782	"	off Mariato Point.	"	**agassizii**, Hartl.
950	"	Port Jackson.	"	*spinicirra* Carp.

In Tiefen von über 1000 *Faden,* 3 Arten.

1350	Faden,	Fiji.	*Antedon*	*acutiradia* Carp.
–1350	"	"	"	*breviradia* Carp.
1600	"	Südsee.	"	*bispinosa* Carp.

Antedon agassizii n. sp.

Taf. I. Fig. 4, 7, 8; Taf. II. Fig. 16, 18, 19; Taf. III. Fig. 23; Taf. IV. Fig. 26.

Centrodorsale von mässiger Grösse, kuppelförmig, am dorsalen Ende cirrusfrei und mit kleinen Dornen bedeckt; 15–22 dünne, namentlich in der äusseren Hälfte stark comprimirte Cirren von etwa 40 mm. Länge; dieselben stehen in 2 und stellenweise 3 Horizontalreihen und bilden bisweilen auch Verticalreihen. Grösste Anzahl der Cirrusglieder etwas über 60; davon die beiden ersten kurz, das dritte länger, das 4. mal solang als das dritte, das 6. am längsten und an langen Cirren 2½ mm. messend; von ihm ab nimmt die Länge der Cirrusglieder rasch ab bis circa zum 20. Gliede, auf welches eine Reihe kurzer, allmälig kleinerwerdenden Glieder folgen; im Verlaufe dieser letzteren ist die dorsale Kante des Cirrus deutlich gezackt. Manche Cirren haben an den letzten 30 Gliedern einen ausgesprochenen Dorn. Dorn des vorletzten Gliedes mitunter schwach.

Erste Radialia eben sichtbar; die zweiten kurz, seitlich vollkommen frei, und für das rhombische Axillare, mit welchem sie einen schwachen Buckel bilden, nicht eingeschnitten. *Axillare* rhombisch, distalwärts stark verbreitert und hier zuweilen mit vertikal abgeplatteter Seite den Nachbaraxillarien angelagert. Zweites Radiale und Axillare sind mit kurzen stumpfen Dornen besetzt. Manchmal sind einzelne 2-gliedrige Distichalserien vorhanden.

10–12 *rundliche schlanke Arme* von beträchtlicher Länge und ganz rauher Oberfläche. Die Armglieder bis zur zweiten Syzygie tragen auf ihrem proximalen und distalen Rande kleine aufrechtstehende stumpfe Dornen nach Art derer auf den Radialien.

Erste Brachialia ziemlich kurz, in der hinteren Hälfte ambulacralwärts verbreitert und hier mit vertical abgeplatteter Seitenfläche dem ersten Brachiale des Nachbarradius angelagert. Diese Seitenfläche bildet die Fortsetzung der des Axillare und somit eine Gesammtfläche mit dieser. — Auf der Innenseite berühren sich die ersten Brachialia in ihrer proximalen Hälfte.

Zweite Brachialia etwas länger als die ersten und distalwärts verbreitert. Sie sowohl als die 3. Brachialia stehen in enger Berührung mit dem 2. und 3. Brachiale des Nachbararms; diese Berührungsstelle ist, da sie ganz ambulacralwärts liegt, schwer zu bemerken. Sie ist von der gleichen Contactfläche des ersten Brachiale durch eine Lücke getrennt. — Die auf das 3. folgenden 7 Glieder sind etwas breiter als lang und in der Mitte eingeschnürt. Vom etwa 9. an nimmt die Länge der Armglieder zu, und ihre Form beginnt mehr dreieckig zu werden, und so bleibt sie ungefähr vom 20. bis zum 30. Gliede. Vom etwa 23. Gliede an (2½ mm.) nimmt die Länge wieder allmälig ab. Auf die dreieckige Gliedform folgt eine mehr trapezoide, und in der zweiten Armhälfte erschienen die Glieder dütenartig in einander gesteckt (Taf. II. Fig. 16). Die distalen Ränder sämmtlicher Armglieder sind stark aufgeworfen und in der zweiten Armhälfte fein dornig. Hier gehen von ihnen Längsriefen aus, welche über die distale Gliedhälfte hinziehen.

Die Dicke der Arme nimmt ganz allmälig ab; die Armlänge beträgt bis 170 mm.; die Zahl der Armglieder bis zu 130.

Erste Syzygie im 3. Brachiale; zweite vom 13.–15., dann in Abständen von 2–3 Gliedern.

Die *Pinnula des zweiten Brachiale* (Taf. II. Fig. 18) ist ziemlich steif, 9–11 mm. lang und zählt 16–18 kurze, glatte Glieder, von denen die ersten 4 — vor Allem aber die 2 ersten — breiter als lang sind. Es ist diese Pinnula an ihrer Basis seitlich abgeflacht und breit und nach etwa dem 6. Gliede spitzt sie sich schnell und fein zu. Ihre äusseren Glieder haben feine, bedornte Distalränder; die Glieder der proximalen Hälfte aber glatte; jedoch sind bei ihnen die ambulacralen Kanten gezackt. Das 2.–5. Glied hat eine Art Kiel; die Verbindung der mehr basalen Glieder unter sich ist eine lockere und dies in erhöhtem Grade an der folgenden Pinnula, deren Basalglieder dementsprechend eine etwas andre Form haben; doch sind auch diese etwas abgeflacht. Es hat das aber schon bei den unteren Gliedern der 3. Pinnula, die rundlicher ist, ein Ende.

Die *Pinnula des* 4. *Brachiale* ist kürzer und hat etwas weniger Glieder, auch ist sie weniger steif und bedeutend feiner gebaut; sie hat wie die folgenden Pinnulæ vorspringende, feine bedornte distale Ränder.

Die dann kommenden Pinnulæ werden bei etwas abnehmender Länge (geringstes Maass 6 mm.) noch zierlicher und ihre Glieder werden länglicher. Vom 14. Brachiale nimmt die Länge der Pinnulæ wieder zu bis zu einem Maximum von 23 mm., und 22–23 Gliedern. Diese letzteren werden bedeutend länger als breit mit Ausnahme der 2 ersten und besonders des Basalgliedes, welches kurz und breit bleibt und eine trapezoide Form hat.

Die *Pinnula des* 3. *Brachiale* ist etwa 2 mm. kürzer als die des zweiten und entschieden schwächer.

Ambulacralfläche der Arme und Genitalpinnulæ deutlich getäfelt. Die Pinnulæ haben Seitenplatten (Taf. 2, Fig. 19).

Sacculi klein und wenig auffallend; mit den Seitenplatten alternirend.

Scheibe stark eingeschnitten; vollständig getäfelt. 9 mm. Dm.

Färbung in Spiritus gleichmässig grünlich gelb, zum grössten Theil durch den stark ebenso gefärbten Alcohol ausgezogen.

Klafterung bis 35 cm.

Fundorte Station No. 3357, nicht weit von Cap Mariato Point (Bucht von Panama), in 782 Faden, "Modern Greensand"; Bodentemperatur 38,5 F. — Station No. 3408, Galapagos, 684 Faden, Globigerinen Schlamm; Bodentemperatur 39,5 F. — Station No. 3409, Galapagos, 327 Faden, Schwarzer Sand; Bodentemperatur 42,3 F.

Bemerkungen: —

Station No. 3408 lieferte ein kleines 11-armiges Exemplar. Es ist dadurch ausgezeichnet, dass seine erste Pinnula weniger glatte Gliedränder hat, als es die Regel ist. Sie sind fein bedornt, was bei den übrigen Exemplaren nur an den äussersten Gliedern der betr. Pinnula der Fall ist.

Station 3408 ergab zwei noch kleinere Exemplare (Armlänge 4½ cm.) und 2 ganz winzige, bei denen das erste Radiale vollkommen sichtbar ist und stumpfe, kurze, aufrechtstehende Dornen besitzt.

Bemerkenswerth für die neue Species ist neben dem Dimorphismus in der Anzahl der Arme, *die Beschrenkung der wandartigen interradialen Contactflächen auf das Axillare und erste Brachiale,* und der Umstand, dass dieselben sogar vollkommen fehlen können. Die 2. Radialia sind seitlich ganz frei; sie berühren sich nur an den Aussenkanten ihres verbreiterten proximalen Endes. Zwischen dieser Berührungsstelle und dem Contact der Axillaria befindet sich ein offner Zwischenraum, ein Loch. Aehnlich scheint sich *Antedon multispina* Carp. zu verhalten, doch ist der Autor geneigt bei ihr dies Vorkommen als Jugendcharakter zu deuten. ["The 2 outer radials, especially the second, which are very short can hardly be described as wall-sided," Chall. Rep. XXVI. p. 117.]

Zu beachten ist sodann die Klafterung von *Antedon agassizii,* welche mit 35 cm. die fast aller andern Arten der Gruppe weit übertrifft. Am nächsten kommt ihr *Antedon valida* Carp. mit "25 cm. spread." Uebertroffen wird ihre Grösse durch *Antedon flexilis* (Basicurva und Spinifera Gr.) mit "55 cm. spread" und *Antedon robusta* (Spinifera Gr.) "spread nearly 50 cm."

Entschiedene Aehnlichkeit verbindet die Art durch die Bedornung ihrer Radialia and unteren Brachialia mit *Antedon bispinosa,* bei welcher die Kelchglieder und die untersten Brachialia der Abbildung nach zu urtheilen (Chall. Rep. XXVI. Pl. 20) nicht nur an den Rändern sondern auf ihrer ganzen Oberfläche mit kleinen stumpfen Dornen besetzt sind. Bei *Antedon agasiizii* geht indessen dieser Schmuck weiter den Arm hinauf als bei jener Art und die kleinen ganz aufrechtstehenden Dornen sind auf den proximalen und distalen Rand eines jeden Gliedes beschränkt, in der Weise, dass jede Gliedverbindung durch 2 Querreihen von Dornen gekennzeichnet ist.

Bezüglich das Calyx verweise ich auf Tafel 1, Fig. 4, 7, 8. — Die Cirrusgruben sind ziemlich gross und besitzen einen ansehnlichen Gelenkkopf. Die ventrale Fläche des Centrodorsale ist annähernd pentagonal und die 5 Ecken ragen etwas vor. Die dorsale Fläche des radialen Pentagons zeigt Spuren eines Basalsterns. Im Trichter erkennt man, etwas tiefer als der dorsale Rand gelegen, die sogenannte Rosette. Die Gelenkflächen der Radialia (Fig. 8) lassen eine Anzahl Gruben erkennen, von denen die zunächst ventralgelegenen Muskelgruben sind. Sie haben annähernd dieselbe Grösse, wie die durch eine schräge Leiste von ihnen getrennten Ligamentgruben und besitzen eine glatte Oberfläche.

Die ambulacrale Täfelung der Pinnula (Taf. 2, Fig. 19, Taf. 3, Fig. 23) besteht aus Seitenplatten und Deckschuppen. Die Deckschuppen sind ganzrandige, feinmaschige, annähernd ovale Blättchen; sie ruhen auf den Seitenplatten und sind wahrscheinlich beweglich, da ihre Stellung eine sehr wechselnde ist. Bald liegen sie dachziegelartig flach übereinander, bald sind sie mehr aufgerichtet; betrachtet man sie in dieser Lage von unten, so sieht man, dass sie eine alternirend schräge Stellung zur Längsaxe der Pinnula haben. Ihre genaue Befestigungsart zu bestimmen ist mir einstweilen nicht gelungen. Die Elemente sind äusserst klein, sehr zerbrechlich und schwer zu isoliren. Sie werden durch Weichtheile festgehalten, die selbst nach mehrere Minuten langen Kochen in Kalilauge nicht zerstört werden. Allzu langes Kochen in Kalilauge vertragen, die sehr dünnen zerbrechlichen Plättchen andrerseits auch nicht. Bezüglich der Seitenplatten sei bemerkt, dass sie im Zickzack stehen. Sie bilden so auf jeder Seite der Ambulacralrinne eine Art spanischer Wand. In den Nischen mehr oder minder verborgen, liegen die Sacculi. Die vorspringenden Ecken werden meist von 2 aneinander stossende Platten gebildet (Fig. 19 und 23 se, se′); an anderen Stellen, so namentlich am Ende einer Pinnula, macht es aber den Eindruck, als ob sie von der Abknickung oder Biegung ein und derselben Platten herrührten. — Die Seitenplatten sind weitmaschiger als die Deckplatten, oft von annähernd viereckiger Gestalt und ausgezackten Rändern. An einigen Stellen sieht man (Taf. 2, Fig. 19), dass durch diese regelmässig wiederkehrenden Auszackungen 2 Lücken in der Berührung der Kanten entstehen. Die oberen dieser Lücken (Fig. 19 s.) werden durch die Sacculi ausgefüllt, durch die unteren (Fig. 19 t.) treten die Tentakel nach aussen.

Die beschriebene Species *Antedon agassizii* n. sp. ist, wie wir gesehen haben, dimorphisch, d. h. sie kommt nicht nur in 10-armigen Exemplaren vor sondern auch nicht selten in solchen, die 11 oder 12, ja vielleicht nochmehr Arme besitzen und diese Exemplare würden, da sie 2 Distichalia haben, zu einer ganz andern Serie der *Carpenter*schen Eintheilung gehören, nämlich in die *Spinifera Gruppe.* Da *Antedon agassizii* darin nicht vereinzelt dasteht, sondern innerhalb derselben Gruppen *Antedon flexilis, duplex, lusitanica* sich ganz gleich verhalten, ebenso *Antedon multispina*, nur mit dem Unterschiede, dass sie auf Grund ihrer 3 Distichalia zum Theil in einer noch andern Serie figurirt, so wirft sich hier naturgemäss die Frage auf, ob eine Eintheilung, die derartige Doppelplacirungen nothwendig machte, natürlich und practisch war. Unserer Auffassung nach hat *Carpenter* in der Zusammenfassung der 10-armigen Antedons zu einer Serie einen entschiedenen Fehler gemacht. Er hat Arten ohne alle sonstige Gemeinschaft miteinander darin vereinigt wie z. B. die äusserst verschiedenen Arten der Basicurva und Tenella Gruppe, während er andre die durch den Besitz einer ambulacralen Bewaffnung, durch ihre horizontale und bathymetrische Verbreitung im Gegensatz zu den übrigen Species stehen, weit voneinander trennte. Auch unter den Arten mit unbedeckten Ambulacren finden sich solche die bald 10-armig, bald mehrarmig erscheinen; auch sie bestätigen, dass der Character der 10-Armigkeit kein zuverlässiger ist und deshalb nicht hätte Veranlassung geben dürfen übrigens soweit verschiedene Species in einer Serie systematisch zu verbinden. Jeden, der sich mit Comatuliden beschäftigt hat, muss der übereinstimmende Charakter im Habitus der Basicurva, Spinifera und Granulifera Gruppe frappirt haben, dazu kommt, dass sie viele Tiefseeformen aus dem pacifischen Ocean und dem Caraibischen Meere enthalten, während die grosse Mehrzahl der übrigen flacheren Gewässern angehören und indischen Ursprungs sind. (So vor Allem die zahlreichen Arten der Savignyi und Palmata Gruppe.[1]) Unsre Ansicht geht also dahin jene 3 Gruppen und die *Elegans Gruppe*, bei welcher die äusseren beiden Radialia im Gegensatz zu allen andern Antedon Arten durch Syzygie verbunden sind, bei welchen aber die Scheibe und die Ambulacralia innerhalb der äussersten Axillaria getäfelt sind, zu einer grossen Serie zu vereinigen und sie einer zweiten Serie gegenüber zu stellen, welche die Gruppen mit ungetäfelten Ambulacren umfasst. Es stellt sich das übersichtlich folgendermaassen dar.

I. Arten mit getäfelten Ambulacren: Serie I.

a) Die 2 äusseren Radialia durch Gelenk verbunden.

10 Arme	Basicurva Gruppe.
	Acœla "
2 Distichalia	Spinifera "
3 Distichalia	Granulifera "

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[1] *Hartlaub, A.*, Beitrag zur Kenntniss der Comatulidenfauna des Indischen Archipels in: Nova Acta Leopold Car. Akad. Bd. LVIII. Nr. 1. 1891. 4o.

b) Die 2 äusseren Radialia durch Syzygie verbunden	Elegans Gruppe.

II. Arten mit ungetäfelten Ambulacren: Serie II.

10 Arme	Eschrichti Gruppe. Milberti " Tenella "
2 Distichalia	Palmata "
3 Distichalia	Savignyi "

II. ARTEN OHNE AMBULACRALE TÄFELUNG.

ESCHRICHTI GRUPPE CARP. CHALL. REP. XXVI. p. 136.

"10 Arme. Die ersten zwei oder drei Pinnula Paare lang und geisselförmig, mit zahlreichen kurzen und breiten Gliedern."

Uebersicht und Verbreitung der Arten: —

Zahl der bekannten Arten: 9.

Bathymetrische Verbreitung, 20–782 Faden.

In Tiefen unter und bis 200 Faden:

20–632	Faden,	Le Have Bank.	*Antedon*	*Eschrichti* Müll.
25–410	"	Smith Sound.	"	*quadrata* Carp.
30	"	Magellan Str.	"	*magellanica* Carp.
75	"	Südsee.	"	*antarctica* Carp.
132	"	Kara See.	"	*barentsi* Carp.
150	"	Südsee.	"	*australis* Carp.

In Tiefen über 200 Faden:

20–632	Faden,	Porcupine Sta. 57.	*Antedon*	*eschrichti* Carp.
25–410	"	Davis Str.	"	*quadrata* Carp.
286	"	Bucht v. Panama.	"	*tanneri* Hartl.
676–782	"	bei Mariato Point.	"	*rhomboidea* Carp.

Die höchste bis jetzt für die Gruppe nachgewiesene *Bodentemperatur* war 35,2 F. (= 1,67 Celsius) [*A. australis* zw. Heard Island und Kerguelen]. Auf Station 286 des Albatross aber — dem Fundort der neuen Art *A. tanneri* betrug die Bodentemperatur 45,9 F. (= 7,70 Celsius).

Antedon rhomboidea CARP.[1])

Taf. I. Fig. 1, 2, 3, 6, 10, 11; Taf. II. Fig. 12, 14, 15, 17; Taf. III. Fig. 24.

Centrodorsale flach kuppelförmig, mit einer cirrusfreien Aushöhlung im Scheitel; circa 60 Cirren in 4–5 Reihen. Cirren gleichmässig dünn, mit etwa 40 Gliedern, die vom 4. bis etwa 10. langgestreckt sind und bis gegen das 20. Glied noch länger wie breit bleiben. Die Cirren sind mit Ausnahme der 7 oder 8 untersten Glieder stark comprimirt. Von der dorsalen oder ventralen Kante betrachtet, erscheinen daher auch die äusseren Glieder noch länglich. Das dorsale Profil der distalen Hälfte des Cirrus erscheint gezackt weil auf dieser Seite die Enden der Glieder vorspringen und selbst die Form kleiner Dornen annehmen. Die Länge der Cirren erreicht zuweilen 6 cm.

Erste Radiale kurz; zweite sehr kurz, seitlich nicht in Berührung unter einander, auf ihrer Verbindung mit dem Axillare eine starke knopfartige Erhebung, die an jüngeren Exemplaren aber kaum angedeutet ist. Axillare ziemlich gross, dreieckig; seine proximale Seite ist bedeutend breiter als die daran stossende distale Kante des 2. Brachiale.

10 *Arme*, die in ihrer proximalen Hälfte ziemlich dick und glatt sind und überall rundliche Glieder haben. Erste Brachialia kurz, auf der Innenseite mit einander nicht in Berührung; auf ihrer Verbindung mit dem zweiten ein starker Knopf; ähnliche knopfartige Erhebungen von schwächerer Entwickelung bemerkt man alternirend seitlich gelegen bis zum 10. oder 11. Gliede. Zweites Brachiale annähernd dreieckig. 3. Brachiale quadratisch (Syzygie); folgende Glieder bis zum 8. (2. Syzygie) von fast derselben Länge aber mehr trapezförmig. Auf das 8. folgen 2 oder 3 kürzere, scheibenförmige Glieder und auf diese ausgesprochen dreieckige. In der äusseren Armhälfte, wo die Glieder feindornige, verdickte überragende Distalränder bekommen, wird die Gliedform wieder mehr trapezoid.

Erste Syzygie im 3. Brachiale, zweite im 8., dritte Syzygie im 13. oder 14. Gliede, seltner schon im 12. oder erst im 15. Dann in Zwischenräumen von 2–3 Gliedern.

Erste Piunula (Br. 2) lang und dünn geisselförmig, mit etwa 50 ausschliesslich kurzen Gliedern; 18 mm. lang; zweite etwas länger und von fast gleicher Form; ihre Glieder sind etwas dicker und meist länglich. Dritte Pinnula kürzer, mit viel weniger, aber bedeutend verlängerten Gliedern; vierte am kürzersten. Die Pinnulæ bleiben am ganzen Arm dünn und ihre Glieder, mit Ausnahme der 2 basalen, stark verlängert. Länge einer Pinnula am circa 59. Armgliede 25 mm.

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[1]) Chall. Rep. XXVI. p. 148. Pl. XII. Fig. 1 & 2; Pl. XXIV. Fig. 1–3.

Sacculi, spärlich.

Färbung, hellbräunlich.

Scheibe, etwa 12 mm.

Klafterung, wenigstens 31 cm. (Exemplar St. 3357), 28–29 cm. Ex. St. 3424.

Fundorte, St. 3357, in der Nähe von Mariato Point, 782 Faden Tiefe, Moderner Grünsand, Bodentemperatur 38,5 F.; und St. 3424, 676 Faden, bei Las Tres Marias, grüner Sand, Globigerinen, Bodentemperatur 38,04 F.

Die obige Beschreibung ist, mit Ausnahme der untersten Pinnulæ, welche zu verstümmelt waren, und mit Ausnahme der Cirren, die leider ganz fehlten, auf das Exemplar von St. 3357 zu beziehen (Taf. 2, Fig. 15). Die Beschreibung der Cirren und Pinnulæ aber wurde nach den Exemplaren von Las Tres Marias entworfen.

Ich habe die obige Schilderung der Art, die ich schrieb im Glauben, dass die vorliegenden Exemplare einer neuen Species angehörten, hier gegeben, weil sie von *Carpenters* Beschreibung und namentlich von seiner Abbildung, Chall. Rep. XXVI. Pl. 12, in vielen Puncten abweicht. Auf *Carpenters* Abbildung ist das Centrodorsale ventral durch eine scharfe grade Linie begrenzt; von den ersten Radialien sind nur die Ecken sichtbar, die zweiten Radialia scheinen sich seitlich zu berühren; sie sowohl wie die ersten Brachialia sind in Uebereinstimmung mit der Beschreibung l. c. p. 148 "deeply incised." Alles das verhielt sich an meinen Exemplaren anders und veranlasste mich zunächst zu einer specifischen Abtrennung. Nachdem ich aber vor Kurzem vom Hamburger Museum ein von *Dr. Rehberg* in der Magellhan Strasse (Smyth's Channel) gesammeltes Exemplar von *Antedon rhomboidea* selbst untersucht habe, bin ich ganz andrer Meinung geworden. Ich glaube, dass die vermeintlichen Unterschiede nur auf Ungenauigkeiten der von *Carpenter* gegebenen Figur beruhen. Das Hamburger Exemplar von *A. rhomboidea* stimmt in jeder Hinsicht mit denen vom Albatross gesammelten. Die Art kommt also sowohl in der Magellhan Strasse als an der pacifischen Seite Central Americas vor. "A similar extensive geographical range from north to south has been observed in the distribution of some of the Mollusks Echini and Starfishes, which extend all the way from the southern extremity of South America to the Panamic region. The course of the northerly current setting along the west coast of South America must of course act as a distributer of the marine fauna of that region" (A. Agassiz, Bull. Mus. Comp. Zool., XXIII. No. 1, p. 75).

Die Exemplare von Las Tres Marias unterscheiden sich nicht unbeträchtlich von dem Exemplar der St. 3357. Hierfür ist vielleicht maassgebend, dass sie aus sehr verschiedenen Tiefen stammen. Die Las Tres Marias Exemplare haben vor Allem einen viel schlankeren gracileren Habitus und erinnern in ihrer Erscheinung mit ihren dicht zusammengelegten, meist gestreckten Armen an eine *Antedon phalangium*, wie sie *Carpenter* (l. c. Taf. XXVIII) abgebildet hat. Die Knöpfe der Armbasis und der Radialien sind nur schwach entwickelt und die alternirend seitlich gelegenen in der unteren Armgegend fehlen ganz. Die Armglieder, namentlich die syzygialen, sind gestreckter.

Die Abbildungen Taf. 1, Fig. 1–3, 6, 10 und 11 geben ein Bild vom Calyx und seinen einzelnen Theilen. Fig. 1 zeigt das radiale Pentagon von der Dorsalseite. Weder von einem Basalstern noch von einer Rosette sind irgend welche Spuren zu bemerken. Die dorsale Oeffnung des Trichters ist weit (Fig, 1), und der Hohlraum im Centrodorsale (Fig. 2 h) dementsprechend. Die Gelenkflächen der Radialia (Taf. 1, Fig. 3 und 10) zeigen im Gegensatz zu *Antedon eschrichti* (Chall. Rep. XXVI. Pl. 1, Fig. 8a) eine gleichmässige Neigung gegen die verticale Hauptaxe; ihr ventraler Rand ist in der Mitte ziemlich stark eingeschnitten; von diesem Einschnitt verläuft eine Verticalleiste gegen das Loch des Centralcanals zu. Die Muskelgruben sind grösser als die Ligamentgruben und von ihnen durch eine ziemlich horizontal gerichtete Querleiste geschieden, die mit 2 mehr oder minder deutlich ausgeprägten Wurzeln entspringt, deren eine von der Verticalleiste, die andre an der Seite des Centralcanals ihren Ursprung nimmt. Auf der Fläche der Muskelgruben bemerkt man schwache, gebogene Querfurchen. Fig. 6 zeigt die ventrale Oeffnung des Trichters und die kleinen in denselben vorspringenden Zacken, die etwas unterhalb des Randes liegen und ventrale Enden von Längsleisten sind, die sich auf der Trichterfläche der Radialia befinden (Taf. 1, Fig. 11). — Diese Verhältnisse wurden an einem älteren und einem viel jüngeren Exemplare von Las Tres Marias untersucht und übereinstimmend gefunden.

Carpenter bildet im Chall. Rep. die besprochenen Theile für *Antedon eschrichti* und *antarctica* ab. Von diesen zeigt *Antedon eschrichti* die meiste Uebereinstimmung mit unsrer Art. Wir finden auch bei ihm auf der ventralen Kante der Radialia den medianen Einschnitt, und scheint es, dass derselbe innerhalb der Gruppe allgemeiner vorkommt, da er noch viel stärker entwickelt ist an der neuen Species, die uns sogleich beschäftigen wird. *Antedon eschrichti* besitzt ferner Querfurchen in den Muskelgruben, aber was ihn wesentlich unterscheidet, ist der Besitz einer Rosette, von der ich bei *Antedon rhomboidea* nichts bemerkte.

An vielen Exemplaren sassen cystenbildende Myzostomen.

Antedon tanneri n. sp.

Taf. I. Fig. 9; Taf. II. Fig. 13; Taf. III. Fig. 20, 22.

Centrodorsale flach gewölbt, mit einer cirrusfreien Grube im Scheitel. Etwa 60 dichtstehende, gleichmässig dünne, comprimirte Cirren, die eine Länge von 33 mm. erreichen. Die Cirren haben grössten Theils längliche Glieder; die letzten davon sind kürzer und haben manchmal einen endständigen kleinen Dorn auf der dorsalen Seite.

Erste Radialia sichtbar, kurz. Zweite kurz und sich distalwärts verschmälernd, nicht in Berührung untereinander. Axillare rhombisch, am Ansatz der Arme beträchtlich verbreitert.

10 *Arme*, die sehr schlank und dünn sind und von Anfang an eine ganz unebene Oberfläche besitzen. Erste Brachialia von einander getrennt, kurz; zweite von unregelmässiger Form und von etwa doppelter Länge; drittes Glied (Syzygie) noch länger. Die folgenden bis zum 7. sind annähernd quadratisch und haben stark eingebogene Flächen. Das 8. Glied hat die Länge des dritten. Das erste und zweite Brachiale haben bisweilen einen kleinen seitlichen Dorn, der bei lezterem dicht vor dem Ansatz seiner Pinnula liegt. Auf das Glied der zweiten Syzygie folgen noch etwa 3 quadratische Glieder, dann wird die Form mehr trapezoid (aber nirgends dreieckig!). Die Glieder verbreitern sich alle etwas distalwärts, und ihre, in der zweiten Armhälfte etwas dornigen, Ränder überragen stark die Basis der nachfolgenden Glieder, was eine grosse Rauhigkeit der Armoberfläche zur Folge hat. Die Arme sind an ihrer Basis rundlich und weiterhin mehr comprimirt; sie verdünnen sich bis etwa zum 15.-20. Gliede rasch und nachher ganz allmälig.

Erste Syzygie im 3. Brachiale, zweite im 8., dritte im 14., die folgenden in Zwischenräumen von 2–3 Gliedern.

Pinnula des zweiten Brachiale 14–16 mm. lang mit 35–44 kurzen Gliedern, geisselförmig und in eine sehr feine Spitze auslaufend. Am Ende der Pinnula ist eine Art Kamm entwickelt (Taf. 2, Fig. 13). Pinnula des 4. Brachialia manchmal etwas länger sonst ebenso. Dritte Pinnula bedeutend kürzer als die zweite und aus einer viel geringeren Zahl länglicher Glieder zusammengesetzt: ohne Kamm am Ende. Vierte Pinnula ebenso lang, dann nimmt die Länge ganz allmälig ab. Die 14 Pinnula ist etwa 10 mm. lang, doch sind die der äusseren Armhälfte wieder einige mm. länger. Alle diese Pinnulæ sind sehr dünn und haben, mit Ausnahme der zwei basalen, stark verlängerte Glieder.

Scheibe etwa 7 mm. Dm., mit zerstreuten Kalkkörperchen. — Mund central.

Sacculi zahlreich an den Ambulacren der Pinnulæ, Arme und Scheibe.

Klafterung durchschnittlich 20 mm.

Färbung, in Alcohol, hell gelblich; Pinnulæ bräunlich.

Fundort, St. 3385, Eingang der Bucht von Panama, 286 Faden, grüner Mud; Bodentemperatur 45,9 F. Zahlreiche Exemplare.

In einiger Hinsicht gleicht diese, neue Art vollkommen der vorigen, so besonders in der Form ihres Centrodorsale und ihrer Cirren, doch lässt ihr ganz anderer Habitus, die grosse Rauhigkeit ihrer Armoberflächen und die Verschiedenheit der radialen Gelenkfläche, die ich an 2 Exemplaren untersuchte, wohl keinen Zweifel an ihrer Berechtigung zu. Bezüglich der Gelenkflächen vergleiche man die Figuren 9 und 10 auf Tafel 1. Wir finden bei unsrer Art an Stelle einer verticalen Längsleiste, eine breite flache intermuskulare Mulde. Solch eine Bildung ist charakteristisch für die Gattung *Actinometra*, bei *Antedon* aber sehr selten (*Antedon disciformis* Palmata Gruppe). Sie gewinnt für unsre Art an Interesse, wenn man bedenkt, dass auch die unteren Pinnulæ durch den Besitz eines Kammes ein *Actinometra* ähnliches Verhalten zeigen. Von den radialen Gelenkflächen wäre weiterhin der ungemein tiefe Einschnitt ihres ventralen Randes zu erwähnen. Zu beiden Seiten dieses Einschnittes liegen die Muskelgruben auf leicht nach aussen gekrümmten Fortsätzen, die in ihrer Form langen Ohren gleichen. Sie haben leicht angedeutete, gebogene Querleisten. Von den Ligamentgruben sind sie durch sehr schwache Leisten getrennt, die ihren Ursprung seitlich vom Centralcanal nehmen, anfänglich vertical verlaufen und dann in einem ziemlich scharfen Winkel schräg nach oben abbiegen.

Das starke Ueberragen der Armglieder (overlapping of the arm-joints), was unsrer neuen Art ein so eigenes Gepräge verleiht, theilt sie in der Eschrichti Gruppe mit *Antedon antarctica* Carp.

An Zierlichkeit des Wachsthums wird sie wohl von Keiner der verwandten Formen erreicht.

An sehr vielen Exemplaren fand ich cystenbildende Myzostomen.

TENELLA GRUPPE Carp. Chall. Rep. XXVI. p. 156.

"10 Arme. Die Glieder der untersten Pinnulæ, welche oft lang und schlank sind, sind länger wie breit und dies oft in hohem Grade."

Die von *Carpenter* in der Tenella Gruppe zusammengefassten Arten, einige zwanzig an der Zahl, haben ausserordentlich weite Grenzen sowohl in ihrer horizontalen als in ihrer bathymetrischen Verbreitung. Es sind unter ihnen zwei (*Antedon hirsuta* und *exigua*) vom 46. Grade südlicher Breite und eine (*Antedon prolixa*) vom etwa 70. Grade nördlicher Breite. Nur sehr wenige Antedon Arten überschreiten überhaupt diese Breiten. Andrerseits ist die Hauptmenge der bekannten Species atlantisch, fünf aber gehören dem westl. Pacific an und eine Art (*Antedon nana*) dem indischen Archipel (Amboina). Die Arten, welche ich hier zu beschreiben habe, sind die ersten aus dem östlichen Theile des Stillen Oceans. Bathymetrisch gehören zur Tenella Gruppe sowohl littorale Species wie z. B. *Antedon nana* Hartl. und die europäische *Antedon rosacea*, als auch *Antedon abyssicola* aus 2900 Faden, der grössten Tiefe, aus der überhaupt Comatuliden gefischt wurden. Die neue Art *Antedon parvula* zeigt viel Aehnlichkeit mit der ebenfalls pacifischen *Antedon alternata* (south of Japan, northeast of New Zealand, Kermadecs, north of Papua) während *Antedon bigradata* n. sp. näher mit der oben erwähnten *Antedon prolixa* von Faeroe Channel und Smith Sound, Kara See, verwandt ist. Wie die übrigen pacifischen Arten, so wurden auch die vom Albatross gefischten in grossen Tiefen gefangen nämlich *Antedon parvula* in 978 Faden und *Antedon bigradata* in 385 und in 555 Faden. Ausser diesen beiden Species erhielt der

Albatross noch stark verstümmelte Exemplare von zwei andern Arten, von denen das eine, wahrscheinlich *Antedon abyssicola,* bei St. 3381 aus der bedeutenden Tiefe von 1772 Faden gefischt wurde.

Antedon parvula n. sp.

Taf. III. Fig. 21.

Centrodorsale annähernd conisch mit schwacher Wölbung nach aussen. 20–30 Cirren, von 5–6 mm. Länge, deren Gliederzahl etwa 20 beträgt. Das 3., 4. und 5. Glied sind stark verlängert. Die folgenden werden schnell kürzer; die langen Glieder sind in der Mitte eingeschnürt. Die distalen Ränder sämmtlicher Glieder springen vor, und die dorsale Contur des Cirrus ist stark gezackt. Das Ende des Cirrus ist etwas comprimirt.

Erstes Radiale etwas kürzer als das zweite und mit leicht eingebogenem distalem Rande; seitlich frei. Zweite kurz, nicht eingeschnitten, ebenfalls seitlich frei. Axillare rhombisch.

10 *Arme.* Erste Brachialia kurz, scheibenförmig, untereinander nicht in Berührung. Zweite fast doppelt so lang, von etwas wechselnder Form (bald mehr dreieckig, bald mehr viereckig). Drittes Brachiale (Syzygie) noch länger; jedes seiner syzygialen Glieder reichlich so lang wie das erste Brachiale. Es folgen vier fast quadratische Glieder und darauf das 8. (Syzygie), das wieder länger ist; dann ausschliesslich trapezoide Glieder, die bald die definitive, nicht unbedeutende Länge erreichen. (Das Armende ist an keinem Arm erhalten.)

Syzygien im 3., 8., 12. und darauf in Unterbrechungen von einem Gliede.

Pinnula des 2. Brachiale sehr dünn; die Glieder sind in der Mitte und gegen das Ende der Pinnula zu ziemlich stark verlängert, und haben vorspringende, fein gezähnte distale Ränder. (Keine war vollständig, eine aber bis zu 15 Gliedern erhalten). Die folgende Pinnula (Br. 6) 6 mm. lang mit circa 15 Gliedern, die der Mehrzahl noch stark verlängert sind. Pinnula des 6. Brachiale kürzer und aus etwa 10 stark verlängerten Gliedern bestehend. Die Pinnula des 8. Brachiale scheint die kürzeste zu sein. Die Glieder sämmtlicher folgenden Pinnulæ sind stark verlängert.

Scheibe nicht sichtbar (auf etwa 3 mm. Dm. zu schützen).

Färbung weisslich.

Klafterung auf 60 mm. zu schützen.

Sacculi nicht deutlich zu erkennen.

Fundort, St. 3363, 978 Faden, Cocos Islands, weisser Globigerinen Schlamm; Bodentemperatur 37,5 F. Ein Exemplar.

Das winzige Exemplar, welches obiger Beschreibung zu Grunde liegt, trenne ich trotz übrigens grosser Aehnlichkeit mit *Antedon alternata* Carp. vorläufig von dieser specifisch ab, weil sich seine Cirren sehr wesentlich von denen jener unterscheiden (Taf. III, Fig. 21, und Chall. Rep. Pl. 22, Fig. 8 & 9). Ich kann aber nicht läugnen, dass mir trotzdem diese neue Art einiges Bedenken macht. Gewisse Uebereinstimmungen, so vor Allem solche in der Form des Centrodorsale, in der Form der Radialia, in der abwechselnden, ein Glied überspringenden Lage der Syzygieen, sind auffallend genug.

Antedon bigradata n. sp.

Taf. I. Fig. 5.

Centrodorsale ziemlich lang conisch, mit abgestumpfter Spitze und etwas gewölbten Seiten. Die Cirrusgruben sind tief (Cirren selbst sind nicht erhalten), etwa 80 an der Zahl und stehen in 5 Feldern, die durch schmale interradiale Zwischenstreifen getrennt sind. Innerhalb der Felder stehen die Cirrusgruben in 4 verticalen Reihen, jede Reihe mit 4 Gruben. Die einzelnen Gruben berühren sich nicht untereinander.

Zwischen Centrodorsale und jedem ersten Radiale befindet sich eine spaltartige Vertiefung. Erste Radialia sichtbar und zwar nicht bloss an den Ecken des Calyx (wie die *A. hystrix*). Zweite Radiale kurz; es steht senkrecht zur Hauptaxe und im rechten Winkel zum Axillare; ebenso steht das erste Brachiale zum zweiten Brachiale. Kelch und Armbasis erscheinen durch diese Stellungsweise auffallend getreppt. Das radiale Axillare ist viereckig, sein proximaler Winkel spitz und lang ausgezogen; die beiden Seitenwinkel nach vorn gerückt.

Erste Brachiale kurz und dorsal kürzer als ventral. Zweite Brachiale dreieckig, drittes ziemlich kurz, dann 4 ebenfalls ziemlich kurze trapezoide Glieder, die auf ihrer pinnulatragenden Seite etwa halb so kurz sind als auf der andern.

Die *Pinnula* des zweiten Brachiale hat etwa 13 Glieder, von denen schon das erste bedeutend länger als dick ist, das zweite aber schon mindestens 2 mm. lang ist. Die Pinnula des 3. Brachiale ist klein und hat ebenfalls ein längliches Basalglied. Pinnula des 4. Brachiale kürzer wie die des 2. mit 9 Gliedern, von denen das Basale ebenfalls länglich ist. An der Pinnula des 6. Brachiale, die aus etwa 9 Gliedern besteht, sind die zwei basalen Glieder kaum noch länger wie breit und auch die folgenden Glieder etwas kürzer.

Scheibe des Exemplars von St. 3404 hat 12 mm. Dm.

Färbung, hell röthlich braun mit weissen Flecken auf den Verbindungen der Pinnula Glieder.

Fundorte, St. 3358, 555 Faden, in der Nähe von Cap Mariato Point, "Modern Greensand," Bodentemperatur 40,2 F.; St. 3404, 385 Faden, zwischen Chatham und Hood Island, felsiger Grund, Bodentemperatur 43,2 F.

Die neue Art ist nahe verwandt mit *Antedon prolixa* Sladen, und mit dieser ist, wie mir *Carpenter* brieflich mittheilte, *Antedon hystrix* identisch. Da mir die *Sladen*sche Beschreibung von *A. prolixa* nicht zugänglich ist, beschränke ich mich darauf auf die Unterschiede hinzuweisen, welche die neue Art im Vergleich mit *Carpenters* Beschreibung von *A. hystrix* darbietet. Besonders ist da hervorzuheben, dass die Cirren von *A. bigradata* in 5 Feldern stehen und innerhalb dieser wieder in Verticalreihen; sodann ist die spaltartige Vertiefung zwischen jedem ersten Radiale und dem Centrodorsale bemerkenswerth. Mehr Gewicht aber ist wohl noch auf die Verschiedenheit zu legen, welche die Pinnula des 2. Brachiale im Vergleich mit der von *A. hystrix* zeigt. Während sie nämlich bei letzterer 15 mm. lang ist und einige 30 Glieder hat "the first six of which are short and nearly square," hat sie bei unserer neuen Art 13 Glieder, von denen schon das erste bedeutend länger als dick ist.

Antedon spec.

Taf. IV. Fig. 25.

Fundort, St. 3381, 1772 fath., Golf von Panama, in der Nähe von Malpelo Island, "Green Mud," Bodentemperatur 37,2 F. Ein Exemplar.

Am vorliegenden Exemplar fehlen die Cirren, und die Arme sind vom 3. Brachiale ab abgebrochen. Von den untersten Pinnulæ sind nur stellenweise einige Glieder erhalten.

Es handelt sich wahrscheinlich um *Antedon abyssicola* oder eine dieser sehr nahe verwandten Art.

Das Centrodorsale ist conisch und trägt etwa 25 Cirrusgruben, die relativ gross sind und dicht aneinander stehen. (*Antedon abyssicola* hat nach Carpenter 15 Cirren). Die ersten Radialia sind wie bei *A. abyssicola* vollkommen sichtbar. Die Ecken schieben sich ein wenig zwischen die zweiten Radialia ein, sodass diese nicht seitlich in Berührung stehen. Dasselbe Verhalten zeigt *A. abyssicola* gelegentlich in ausgeprägtem Maasse.

An dem Exemplar sitzt als Schmarotzer festgeheftet ein *Stylifer* verwandte Schneckenart (nach Prof. *v. Martens* wahrscheinlich eine *Mucronalia*).

Antedon spec.

Taf. IV. Fig. 27.

Fundort, St. 3354, 322 fath., in der Nähe der Küste bei Mariato Point, "Green Mud," Bodentemperatur 46,0 F. Ein Exemplar.

Eine Bestimmung ist nicht ausführbar, weil die Cirren und untersten Pinnulæ fehlen und die Arme sämmtlich nahe ihrer Basis abgebrochen sind. Die conische Form des Centrodorsale, die bedeutende Zahl der Cirrusgruben sowie die Form der Radialia und untersten Armglieder lassen auf eine *Antedon prolixa* nahestehende Art wenn nicht gar auf diese selbst schliessen.

Uebersicht der vom Albatross gesammelten Arten.

Gesammtzahl der Species, 7.

Sämmtliche Species gehören dem Genus *Antedon* an und vertheilen sich, wie folgt, auf drei Gruppen: —

I. BASICURVA GRUPPE Carp.

Antedon agassizii n. sp.

Fundorte:
St. 3357, 782 Faden, "modern greensand," nicht weit von Mariato Point.
" 3408, 684 " Globig. Ooze. } Galapagos.
" 3409, 327 " "black sand." } Galapagos.

II. ESCHRICHTI GRUPPE Carp.

Antedon rhomboidea Carp.

Fundorte:
St. 3357, 782 Faden, "modern greensand," nicht weit von Mariato Point.
" 3424, 676 " "gray sand," Las Tres Marias Inseln.

Antedon tanneri n. sp.

Fundort:
St. 3385, 286 Faden, "green mud," Golf von Panama.

III. TENELLA GRUPPE Carp.

Antedon spec.

Fundort:
St. 3354, 322 Faden, "green mud," bei Mariato Point.

Antedon bigradata n. sp.

Fundorte:
St. 3358, 555 Faden, "modern greensand," nicht weit von Mariato Point.
" 3404, 385 " "rocks," Galapagos.

Antedon parvula n. sp.

Fundort:
St. 3363, 978 Faden, "white Glob. Ooze.," Cocos Inseln.

Antedon spec. (abyssicola Carp. ?).

Fundort:
St. 3381, 1772 Faden, "green mud," nicht weit von Malpelo Island.

Besonderes Interesse verdienen des Fundortes wegen die zu *Eschrichti Gruppe* gehörenden Arten.

ERKLÄRUNG DER ABBILDUNGEN.

TAFEL I.

Fig. 1.	**Antedon rhomboidea Carp.**	Exemplar von Las Tres Marias. Dorsale Ansicht des radialen Pentagons; vergrössert × 5.
Fig. 2.	" "	Ventrale Ansicht des Centrodorsale vom selben Exemplar; vergr. × 5.
Fig. 3.	" "	Calyx von der Seite, vom selben Exemplar; vergr. × 5.
Fig. 4.	" **agassizii n. sp.**	Ventrale Ansicht des Centrodorsale; vergr. × 7.
Fig. 5.	" **bigradata n. sp.**	vergr. × 3.
Fig. 6.	" **rhomboidea n. sp.**	Ventrale Ansicht des Calyx; vergr. × 5.
Fig. 7.	" **agassizii n. sp.**	Dorsale Ansicht des radialen Pentagons vom selben Exemplar wie Fig. 4; vergr. × 7. *r.* Rosette; *b.* ein Strahl des Basalsterns.
Fig. 8.	" "	Seitenansicht des Calyx; vergr. × 6. *m.* Muskelgrube; *l.* Ligamentgrube.
Fig. 9.	" **tanneri n. sp.**	Gelenkfläche des ersten Radiale; vergr. × 15.
Fig. 10.	" **rhomboidea**	Gelenkfläche des ersten Radiale; vergr. × 11.
Fig. 11.	" "	Ansicht eines Radiale vom Trichter aus; vergr. × 6.

TAFEL II.

Fig. 12.	**Antedon rhomboidea** n. sp.	Pinnula des zweiten Brachiale; vergr. × 4.
Fig. 13.	" **tanneri** n. sp.	Ende der Pinnula des zweiten Brachiale; stark vergr.
Fig. 14.	" **rhomboidea** n. sp.	Theil eines jungen Exemplars von Las Tres Marias; vergr. × 4.
Fig. 15.	" "	Theil eines Exemplars von St. 3357; vergr. × 2½.
Fig. 16.	" **agassizii** n. sp.	Stück aus der äusseren Armhälfte; vergr.
Fig. 17.	" **rhomboidea** n. sp.	Cirrus eines Exemplars von Las Tres Marias; vergr. × 2.
Fig. 18.	" **agassizii** n. sp.	Proximale Armregion; vergr.
Fig. 19.	" "	Glied einer Pinnula von der Seite; sehr stark vergr. *se, se′* Seitenplatten; *s.* Platz des Sacculus; *t.* Lücke für den Tentakeldurchtritt; *d.* Deckplatten.

TAFEL III.

Fig. 20.	**Antedon tanneri** n. sp.	vergr. × 1½.
Fig. 21.	" **parvula** n. sp.	Cirrus; vergr. × 11.
Fig. 22.	" **tanneri** n. sp.	Stück aus der mittleren Armgegend; vergr. × 4.
Fig. 23.	" **agassizii** n. sp.	Glied einer Pinnula; stark vergr.
Fig. 24.	" **rhomboidea**.	Exemplar von Las Tres Marias; vergr. × 1½.

TAFEL IV.

Fig. 25.	**Antedon** spec. **abyssicola** Carp.? mit einer daran sitzenden **Mucronalia**?	vergr. × 4½.
Fig. 26.	**Antedon agassizii** n. sp.	vergr. × 2. *Peters* del.
Fig. 27.	" spec. (Tenella Gruppe).	vergr. 4½.

Im Anhang an die Beschreibung der Albatross Crinoiden möchte ich noch einen ebenfalls dem Mus. of Comp. Zoölogy gehörenden Antedon von *Gaspard Str.* beschreiben, den ich für neu erachte.

Antedon subtilis n. spec. (Palmata Gruppe).

Centrodorsale flach scheibenförmig, mit etwa 20 glatten Cirren am Rande, die meist zweireihig stehen. Cirren etwa 12 mm. lang, ziemlich dünn und in der äusseren Hälfte comprimirt. Circa 20 Cirrusglieder, die vom 5. an etwas länglich sind; die äusseren Glieder haben einen ganz schwachen Dorn, das vorletzte einen stärkeren.

Radien vom ersten Radiale an ganz frei seitlich. Erste Radialia seitlich etwas sichtbar. Zweite Radialia kurz, etwas kürzer wie die Seiten des fünfeckigen Radiale axillare. Distichale und palmare Stämme zweigliedrig. Erste Distichale und Palmare etwa so lang wie die Seiten des 2. u. 3. Radiale zusammengenommen. Keine Postpalmaria. Die Glieder der Radialia, Stämme und Arme vollkommen glatt. Nirgends Buckel auf den Verbindungen der Axillaria oder sonstige Protuberanzen.

20 *Arme.* (Ein Radius mit fünf Armen, einer mit drei, die übrigen mit vier). Die Arme sind ziemlich dünn, vollkommen glatt, etwa 5 cm. lang und mit sehr feinen Pinnulæ besetzt; die Armglieder sind kurz und zwar bis zum 8. mehr oder minder scheibenförmig, vom 9. an aber ausgesprochen dreieckig; so bleiben sie bis etwa zum 20. Gliede, von da ab wird die Form mehr trapezoid, und später quadratisch. Das erste Brachiale steht in theilweisem Contact mit dem Nachbargliede.

Erste Syzygie im 3. Brachiale, zweite im 14. Brachiale, die dritte nach einen Zwischenraum von 5–6 Gliedern, die folgenden in Zwischenräumen von 6–7.

Pinnula des 2. Brachiale etwa 7 mm. lang mit circa 13 glatten Gliedern, die mit Ausnahme der 2 basalen länglich sind. Pinnula des 4. Brachiale beträchtlich stärker und 10 mm. lang, geisselförmig. Glieder wie bei der vorigen länglich mit Ausnahme der zwei ersten. Die darauf folgenden 3 Pinnulæ derselben Armseite ganz winzig, etwa 2 mm. lang. Dann nimmt die Länge allmälig etwas zu, doch bleiben die Pinnulæ des Mittelarms klein und dünn. Die Pinnulæ des Armendes sind von haarartiger Feinheit, aber etwas länger (5 mm.), die Glieder stets länglich.

Sacculi dichtstehend.

Klafterung etwa 10½ cm.

Scheibe tief eingeschnitten.

Färbung gleichmässig weiss.

Fundort, Gaspar Str. Ein Exemplar.

Die Art ist nahe verwandt der von mir beschriebenen Art *Ant. Klunzingeri* von Koseir aus dem Rothen Meer, unterscheidet sich aber von dieser durch die Kleinheit der Pinnulæ des 6. Brachiale. Dieselbe ist bei *A. Klunzingeri* so lang wie die des ersten Brachiale. Möglicher Weise wird sich nach Untersuchung grösseren Materials herausstellen, dass die beiden Arten identisch sind.

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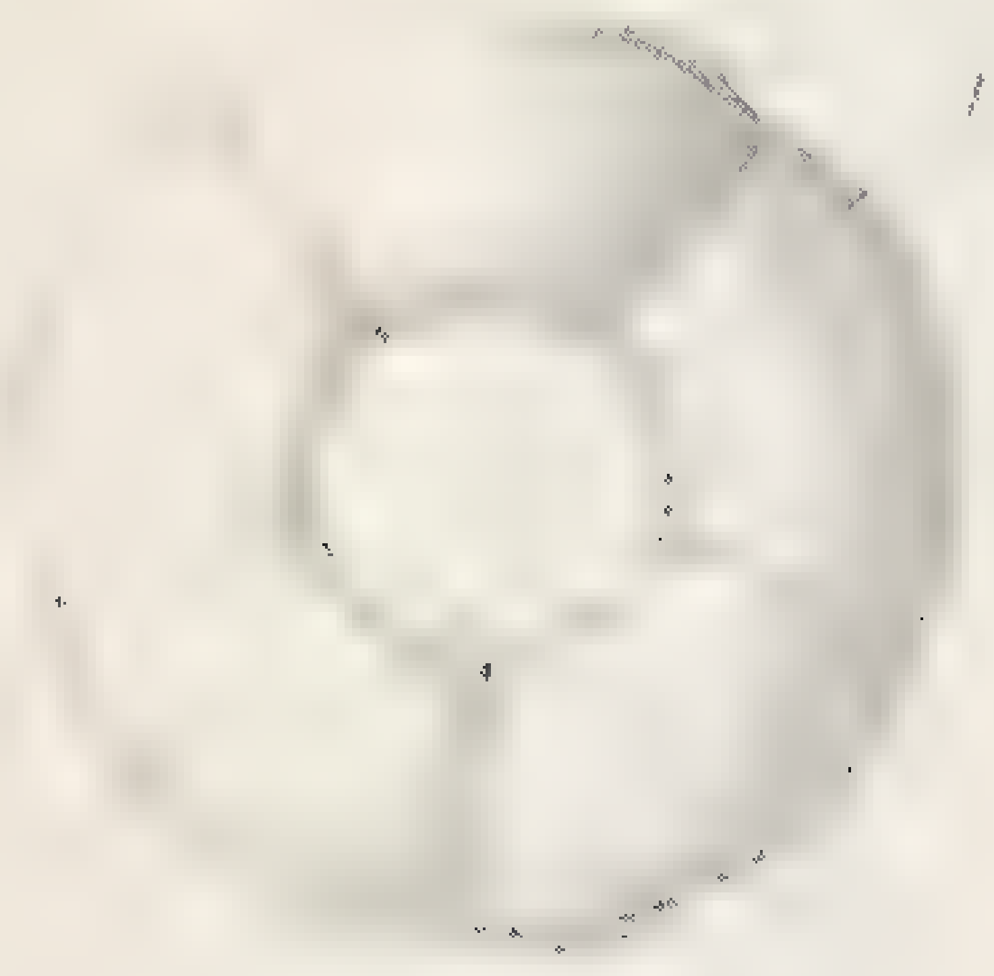

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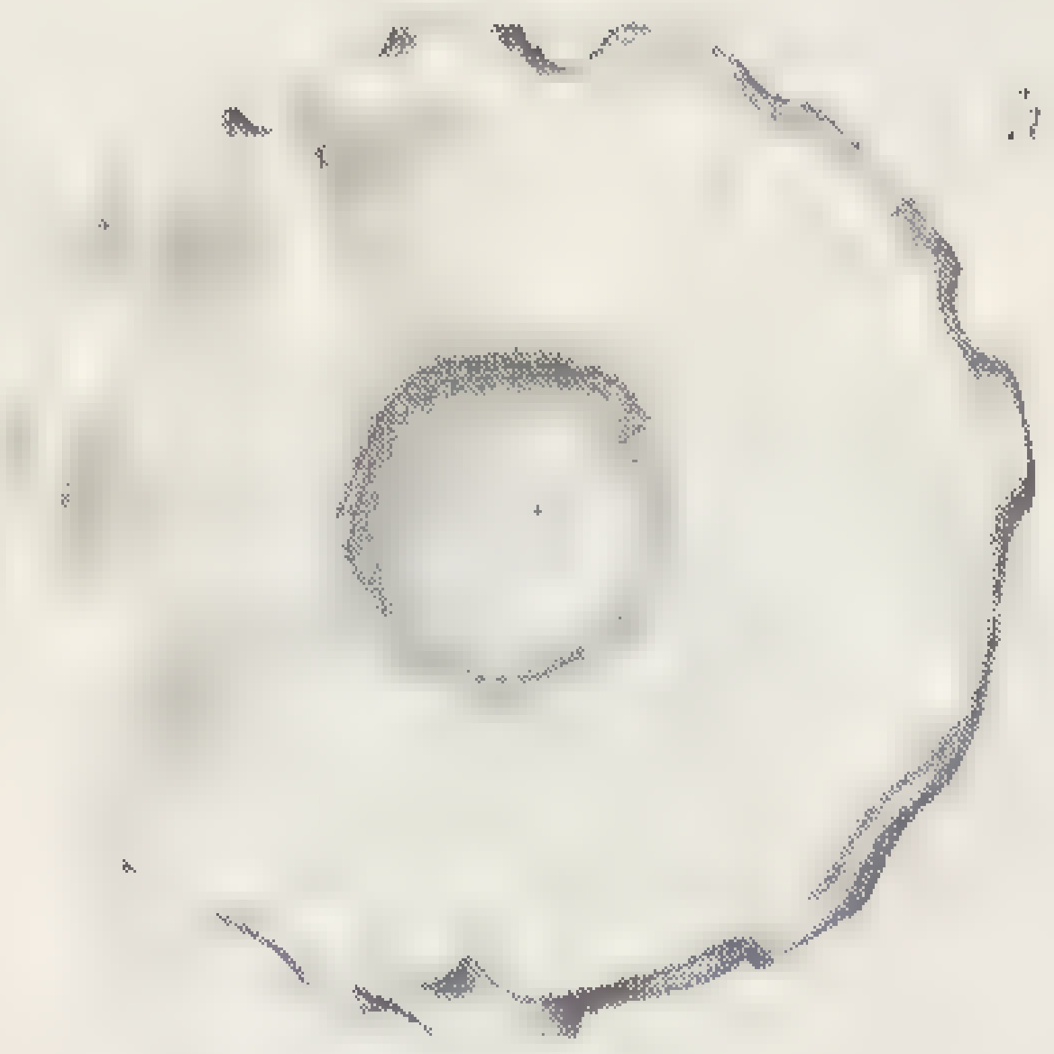

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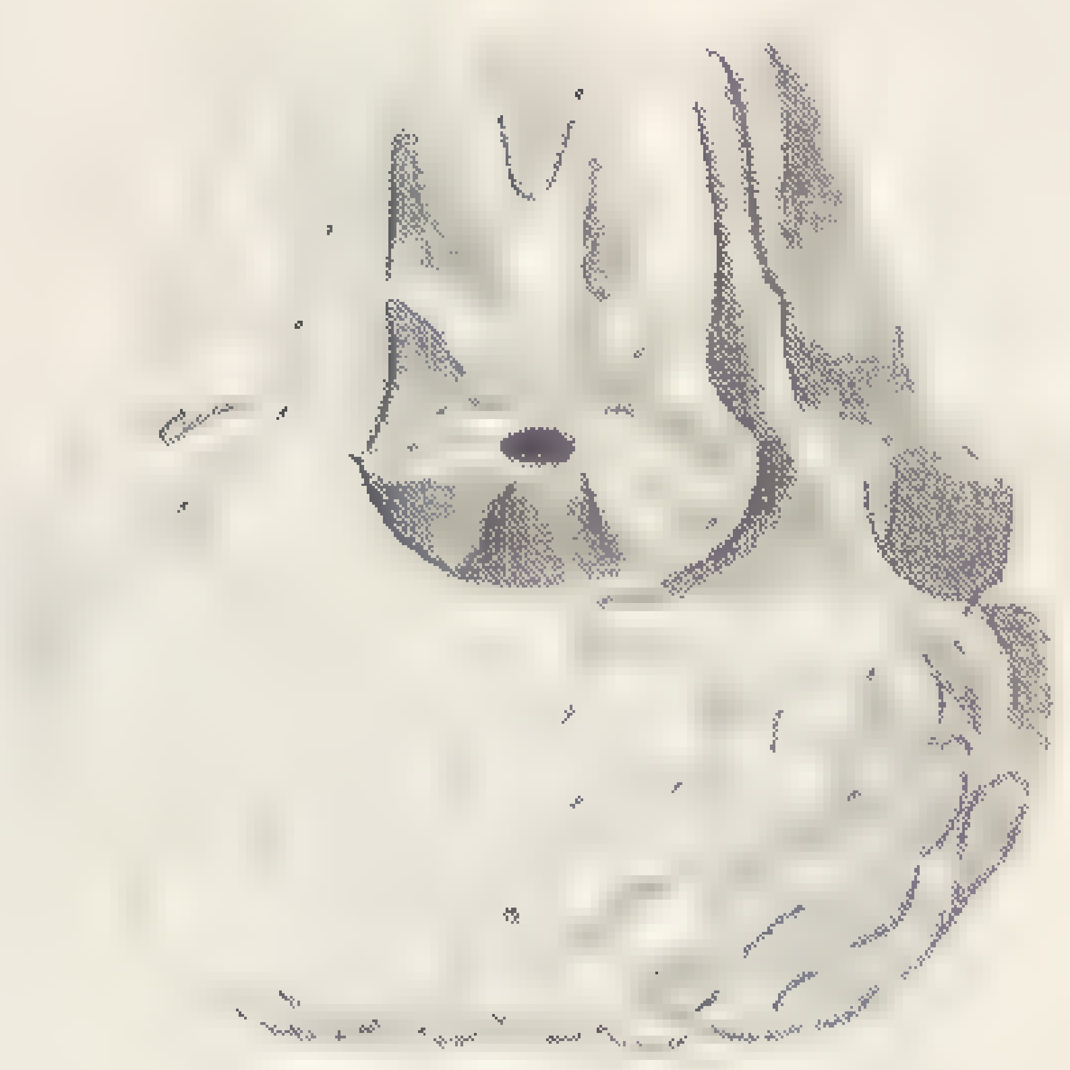

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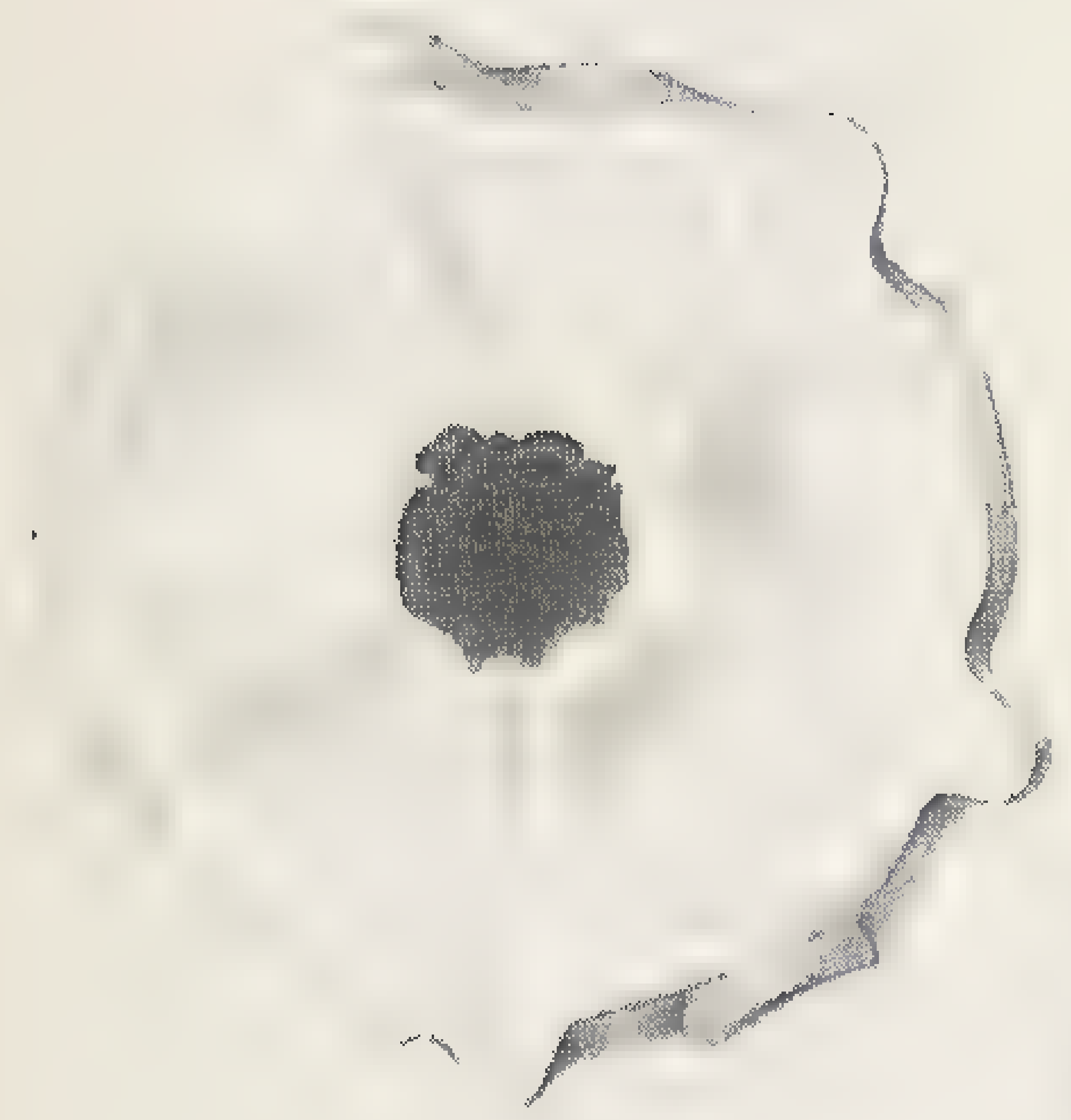

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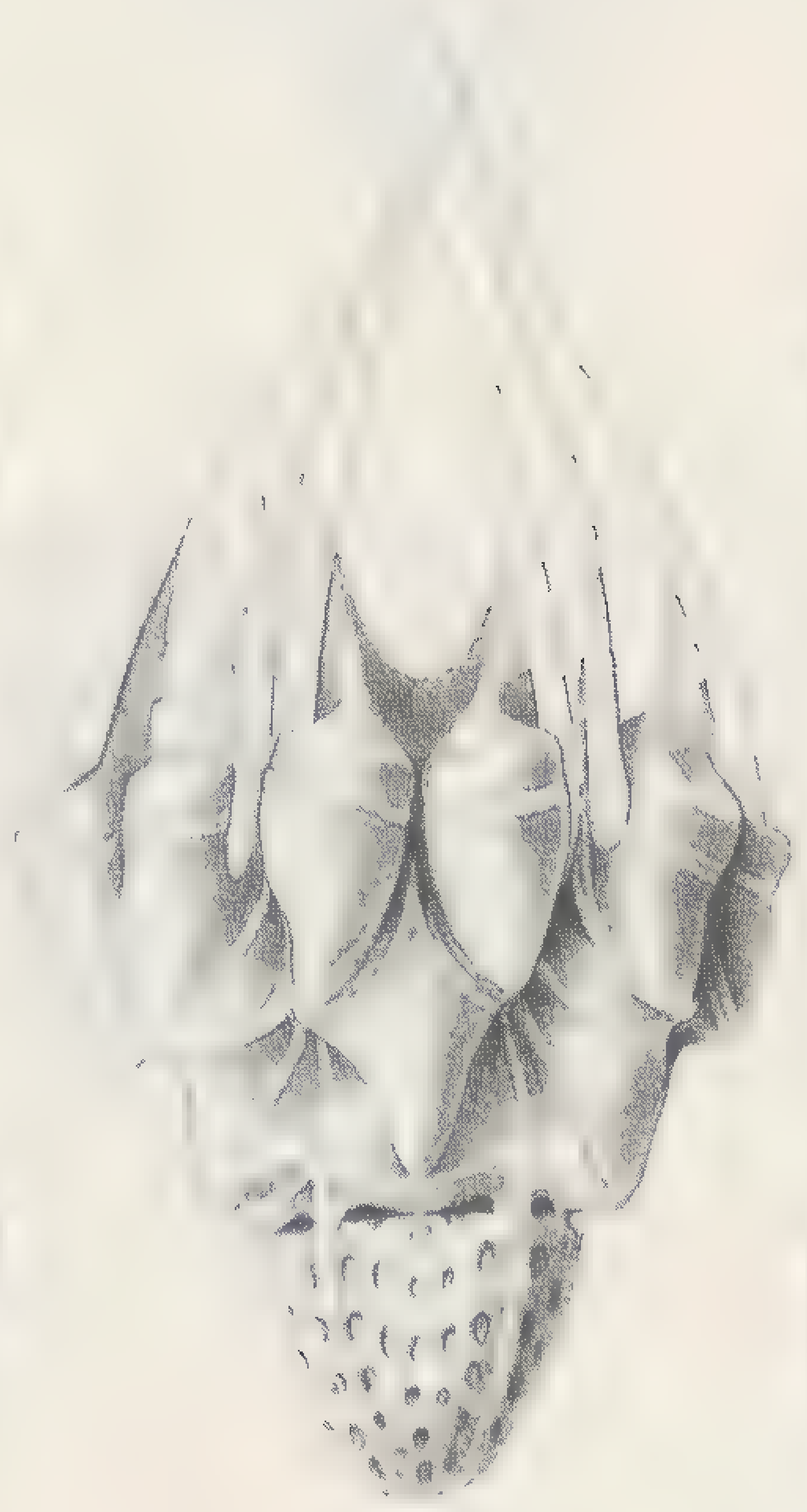

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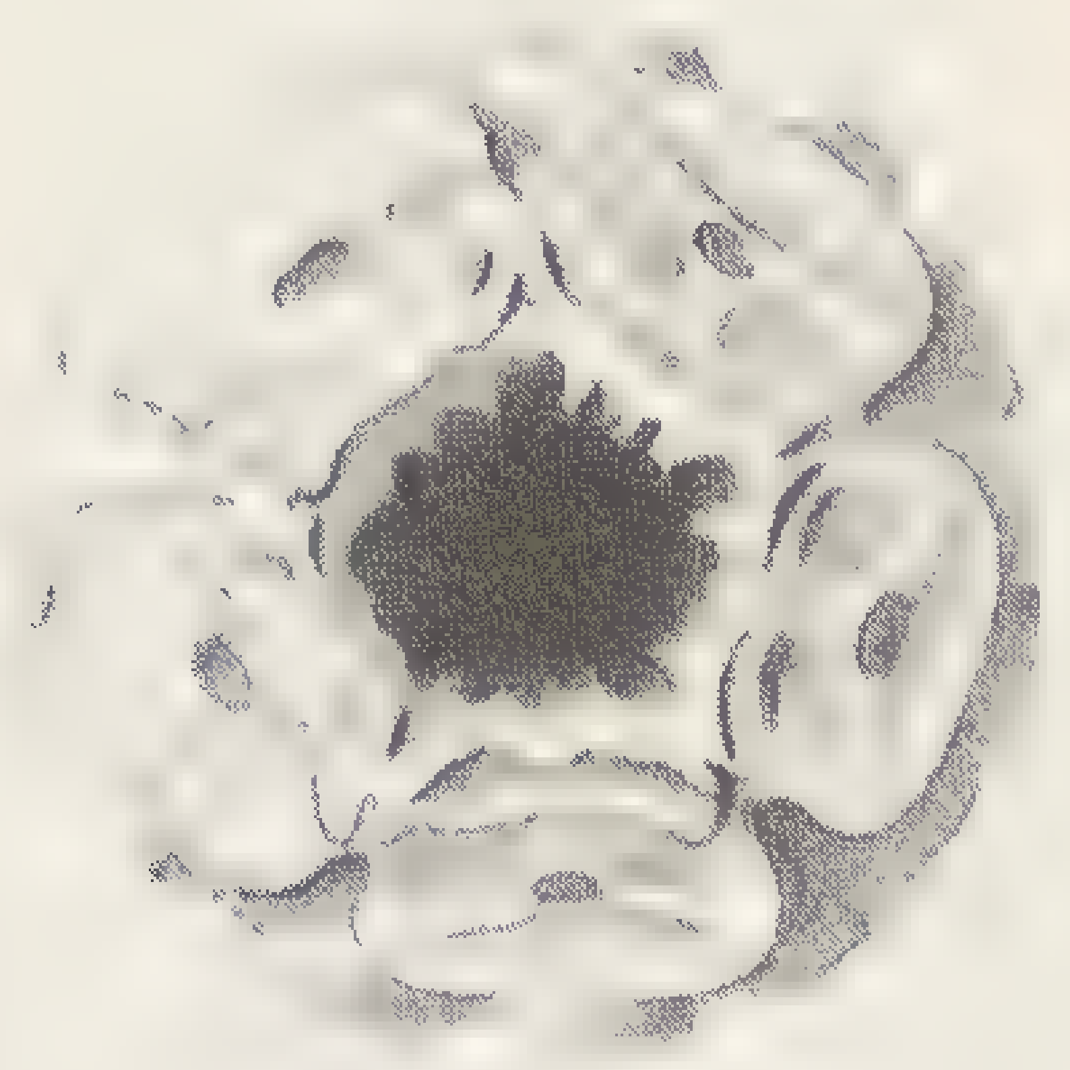

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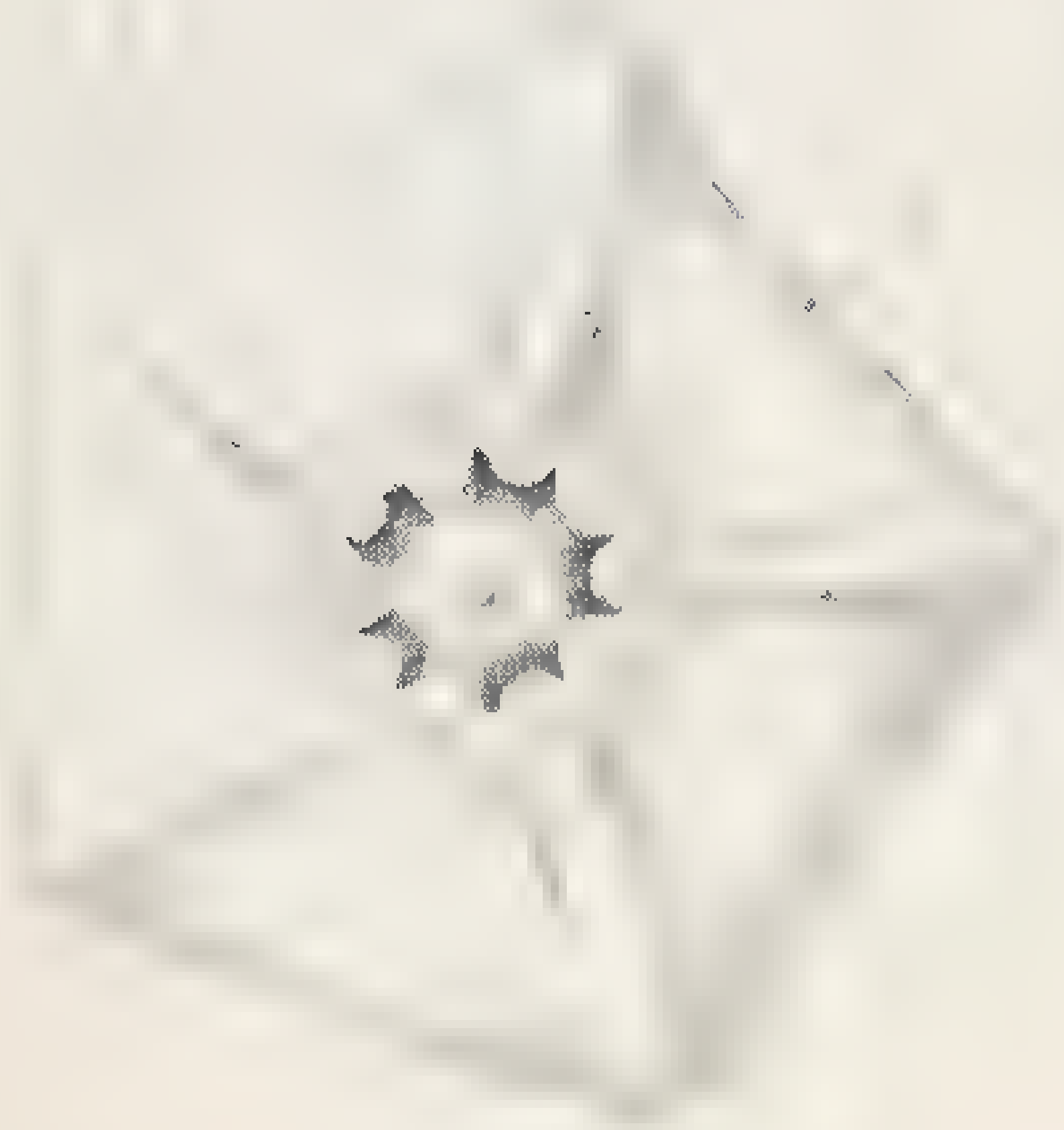

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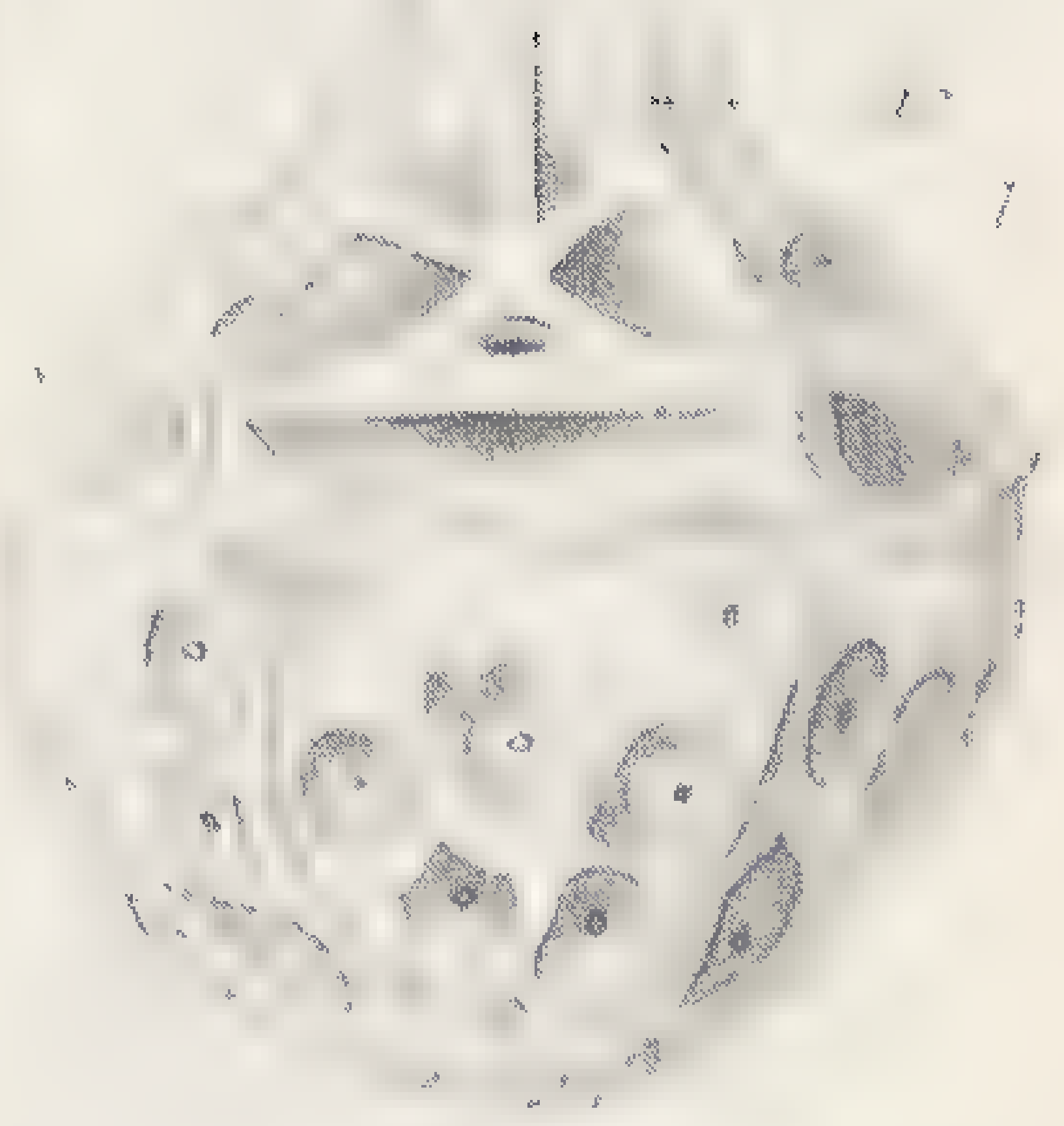

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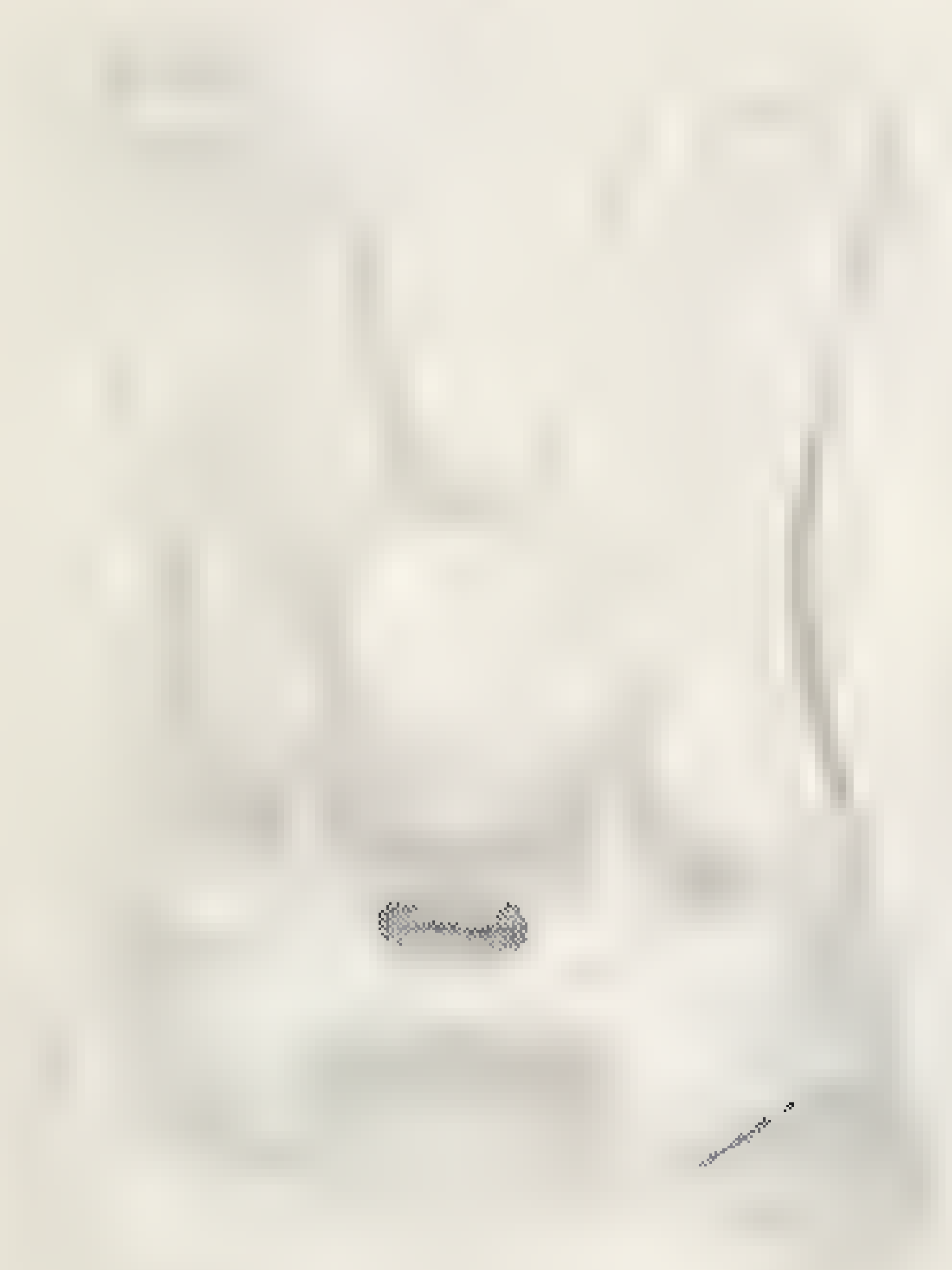

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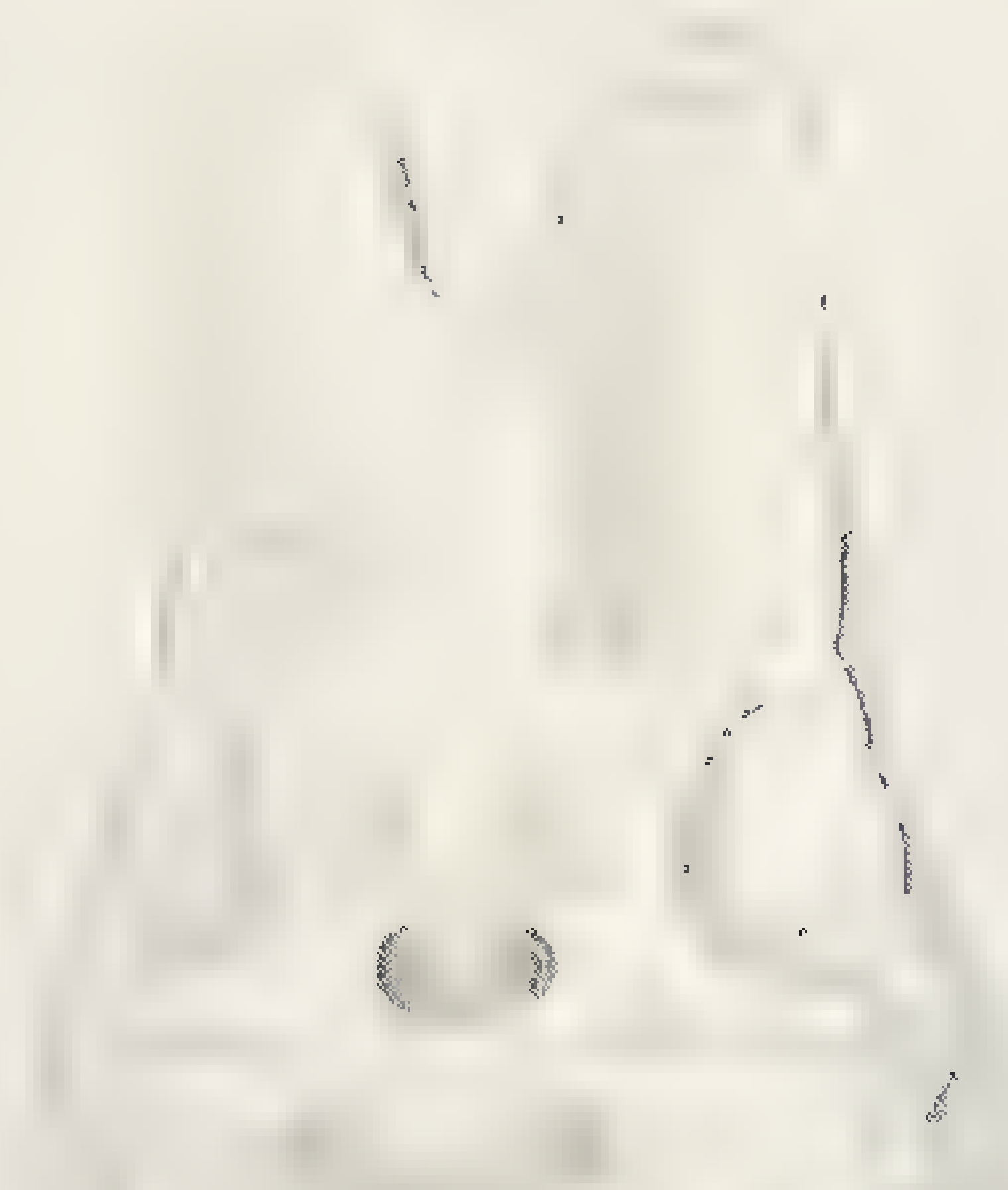

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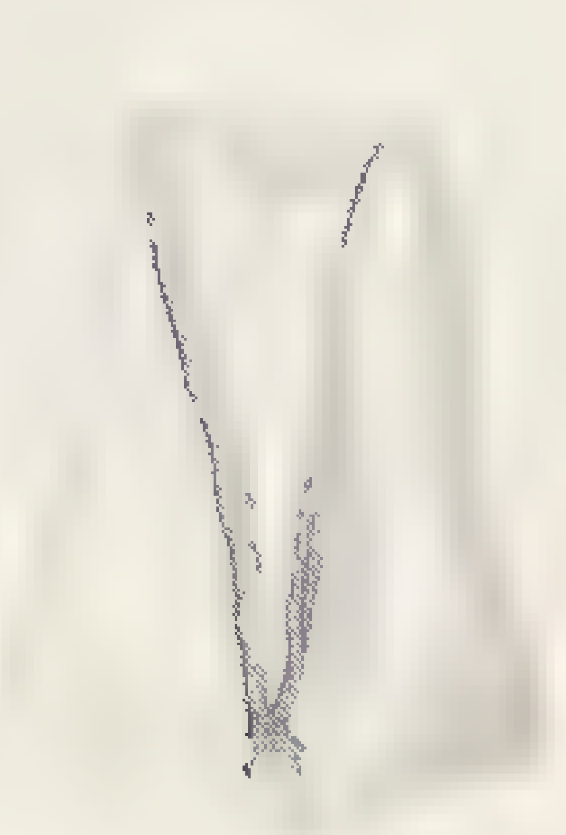

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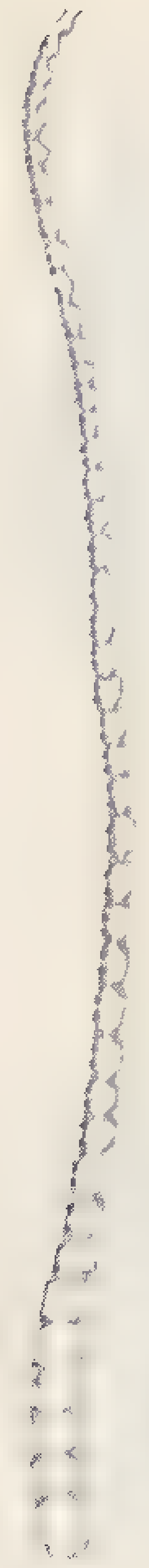

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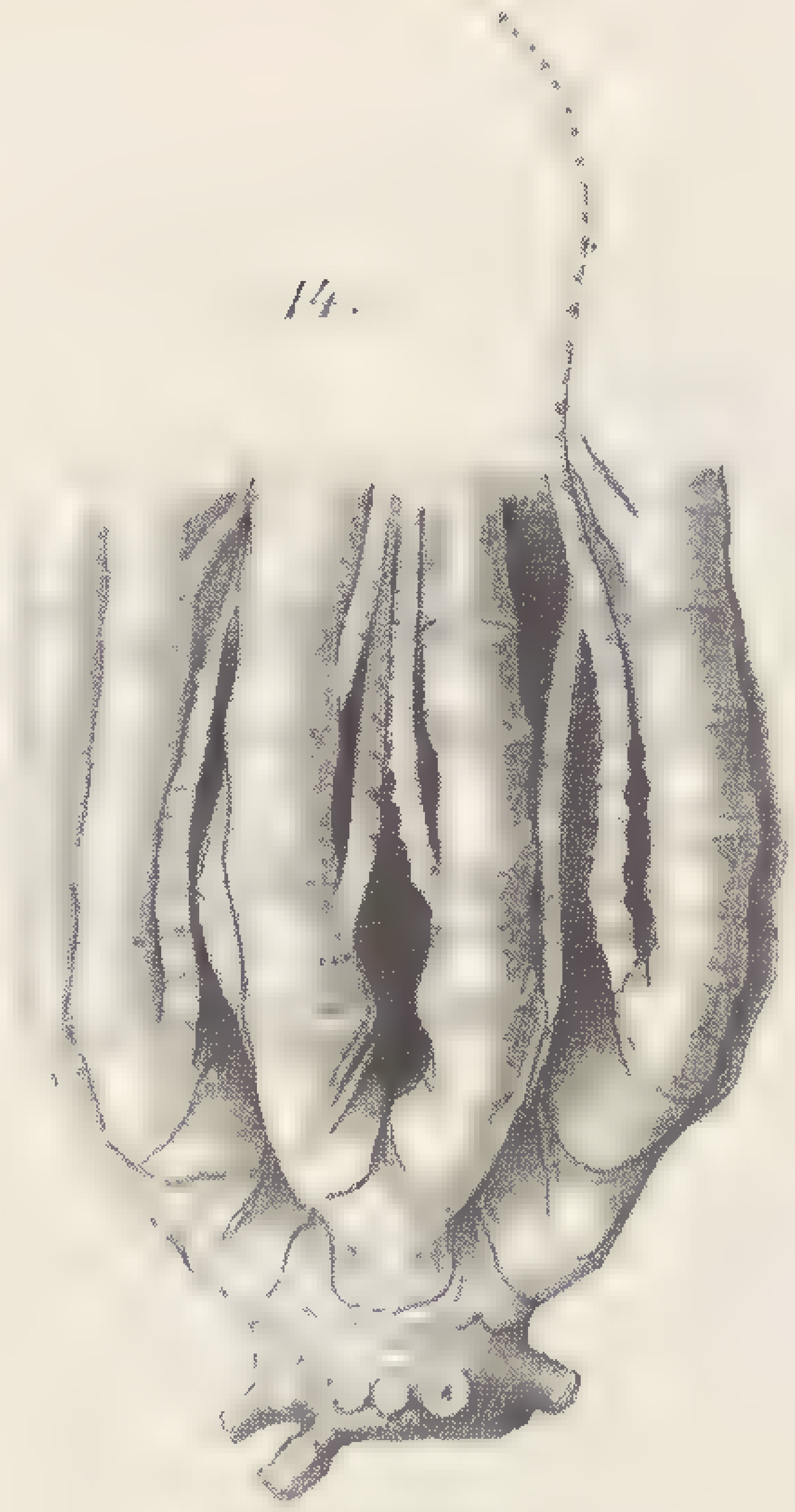

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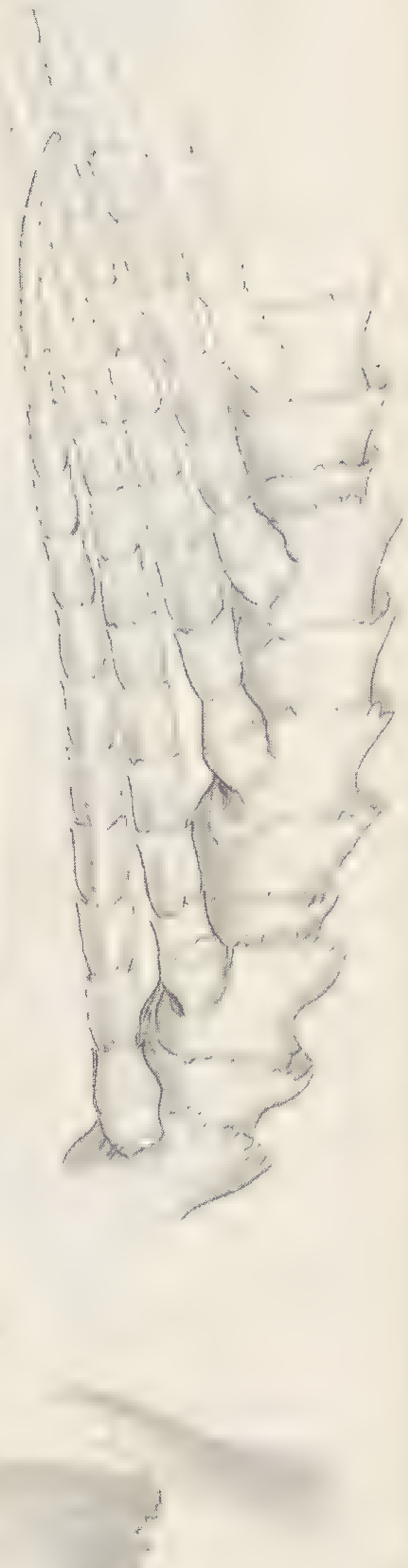

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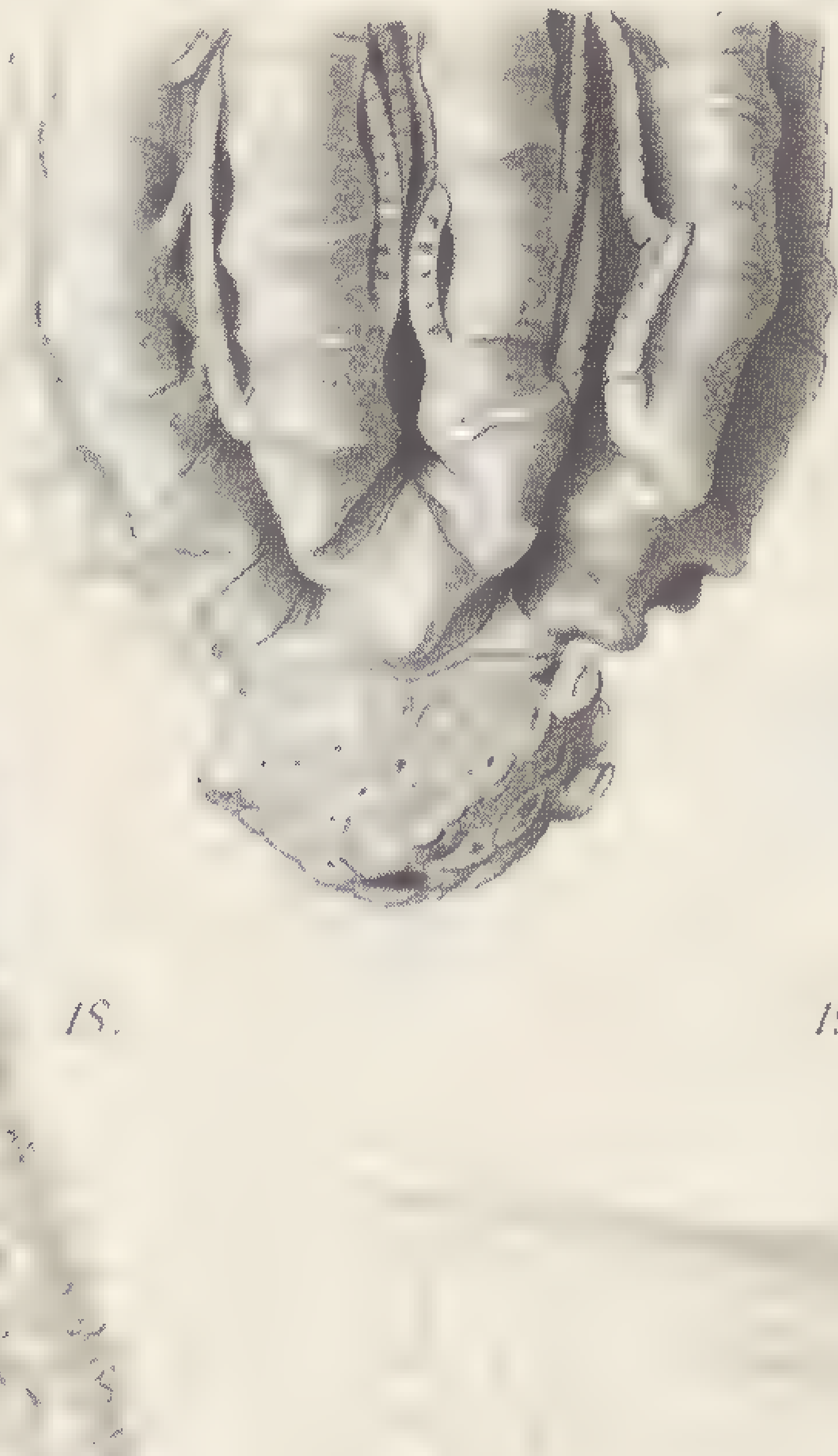

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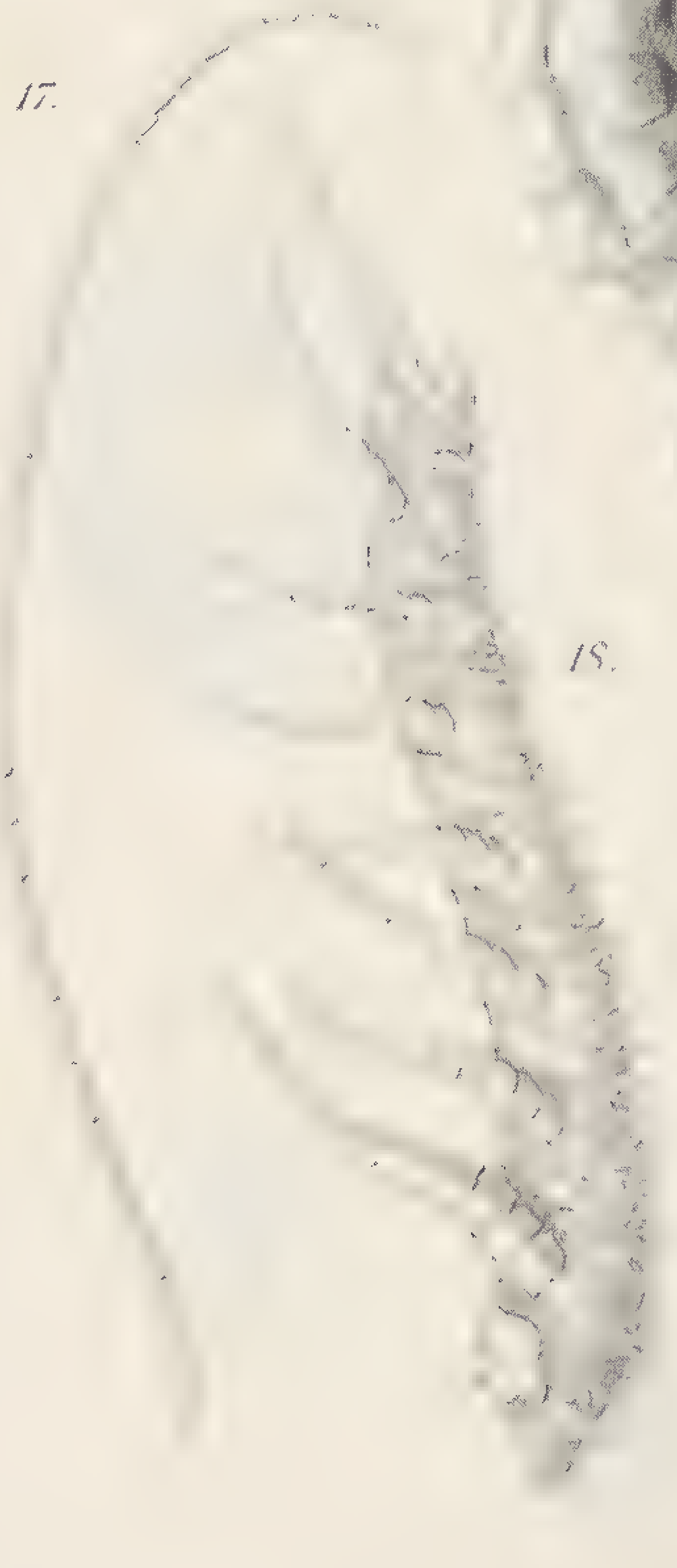

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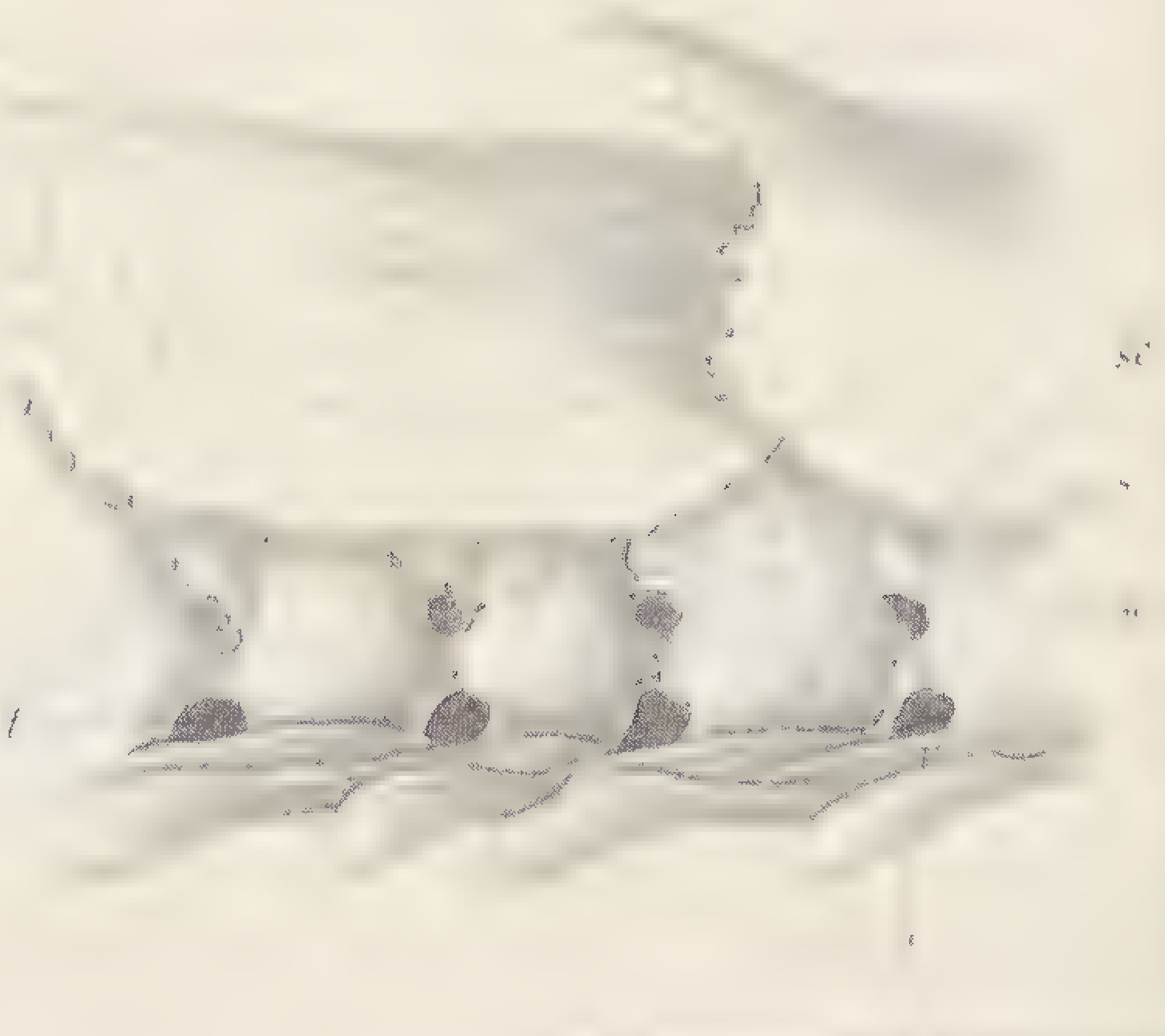

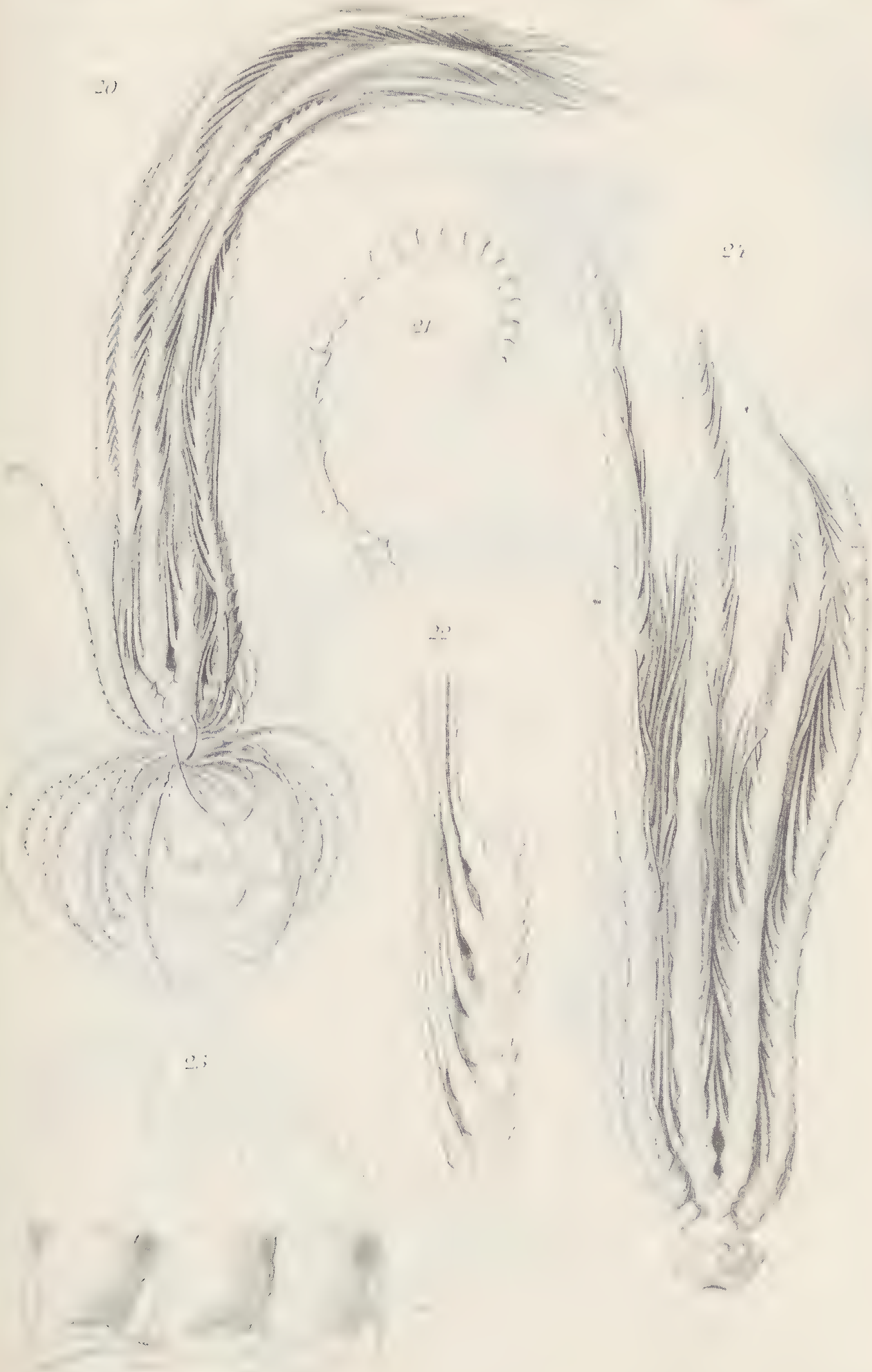
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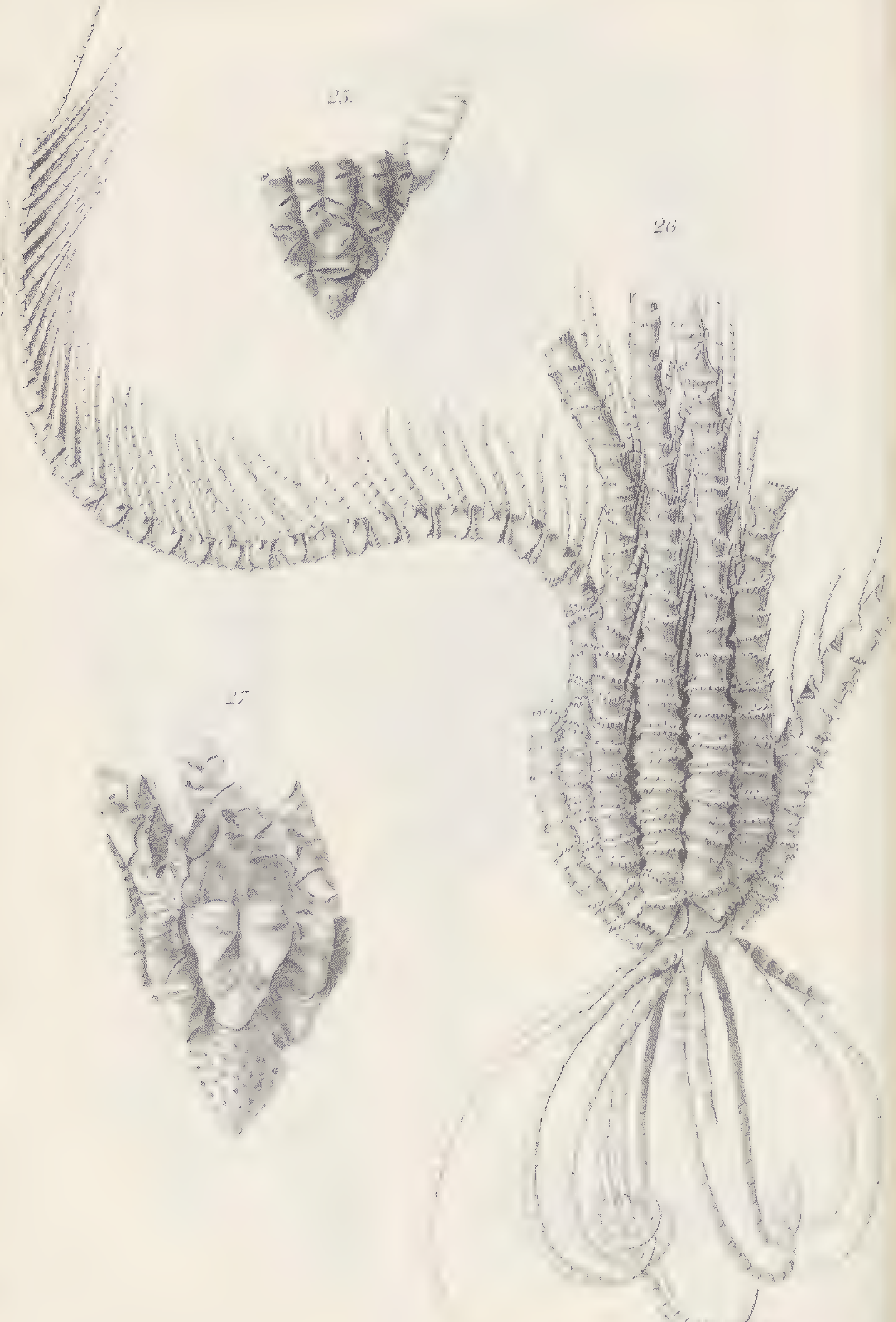

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Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
Vol. XXVII. No. 5.

REPORTS ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

XIX.

DIE OSTRACODEN.

Von G. W. Muller.

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With Three Plates.

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No. 5. — *Reports on the Dredging Operations off the West Coast of Central America to the Galapagos, to the West Coast of Mexico, and in the Gulf of California, in charge of* ALEXANDER AGASSIZ, *carried on by the U. S. Fish Commission Steamer "Albatross," during* 1891, Lieut.-Commander Z. L. TANNER, U. S. N., Commanding.

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XIX.

Die Ostracoden. Von G. W. MÜLLER.

Die Zahl der gesammelten Arten ist nur gering . zwei Cypridiniden und vier Halocypriden, doch knüpft sich an die beiden Cypridiniden als Vertreter einer neuen Gattung von sehr abweichendem Körperbau und ebenso abweichender Lebensweise ein besonderes Interesse. Es handelt sich, was ich zum Verständniss der folgenden Beschreibung vorausschicke, um Arten von ausschliesslich pelagischer Lebensweise. Ich lege der Besprechung der Cypridiniden, für die ich mit Rücksicht auf ihre auffallende Grösse den Gattungsnamen *Gigantocypris* wähle, die häufigere der beiden Arten, *Gigantocypris Agassizii*, zu Grunde.

Bei einer oberflächlichen Betrachtung kann man in Zweifel sein, ob man es mit einem Ostracoden zu thun hat. Zunächst überrascht die ausserordentliche Grösse, wie sie sonst von lebenden Ostracoden nicht entfernt erreicht wird, wie sie sich auch nur ganz ausnahmsweise bei fossilen findet. Die Thiere erreichen eine Länge von 23 mm. bei eine Höhe von 19.5 und eine Breite von 18 mm. Weiter bietet das Thier das Aussehen einer grossen, dünnwandigen, durchscheinenden Blase mit kurzer, schlitzförmiger Oeffnung, nicht den eines Thieres mit zweiklappiger Schale (Tafel 1, Fig. 1, 2). Eigentlich kann man auch nicht mehr von einer zweiklappigen Schale sprechen. Während sonst bei den Ostracoden die Strecke, für welche beide Schalenhälften mit einander verbunden sind, etwa $\frac{1}{3}$, stets aber weniger als $\frac{1}{2}$ der Peripherie beträgt, beträgt sie hier etwa $\frac{2}{3}$, nur $\frac{1}{3}$ der Schalenränder ist frei. Die freien Ränder der Schale legen sich dicht aneinander, nur am vorderen und hinteren

Ende des Schlitzes weichen sie deutlich auseinander, vorn zur Bildung der bekannten Rostralincisur (Tafel 1, Fig. 1, 2, 6, 8), hinten für eine ovale Oeffnung, welche den anderen Cypridiniden fehlt (Tafel 1, Fig. 1, 2, 7, 9). Innerhalb der übrigen ⅔ der Peripherie, wo beide Schalenhälften fest mit einander verbunden sind, zeigt sich nur in der Nachbarschaft der genannten Oeffnungen eine deutliche Abgrenzung zweier Schalenhälften, sonst markirt sich die Grenze nur als feine, schwer aufzufindende Linie.

Uebrigens ist die Schale sehr dünnhäutig, nur die freien Ränder sind verstärkt. Die Schale ist so dünn und zart, dass fast sämmtliche Thiere, die ich erhielt, sehr stark, auf ¼ oder weniger ihres Volums zusammengedrückt waren, wofür freilich nicht nur die geringe Widerstandsfähigkeit der Schale, sondern auch die geringe Menge fester Substanz, das ausserordentliche Ueberwiegen der Bluträume verantwortlich zu machen ist. Vermuthlich ist dieses Zusammendrücken bereits im Netze erfolgt, die Thiere haben dem Druck des Wassers nicht widerstehen können. Nur 1 Individuum war in seiner Körperform gut erhalten, 1 zweites leidlich, alle übrigen waren stark zusammengedrückt.

Von Verkalkung ist an der Schale durchaus nichts zu bemerken; auch von krystallinischen Concretionen, wie man sie in Folge der Auflösung und Wiederausscheidung des Kalkes in der Schale oder an anderen Körpertheilen bei conservirten Halocypriden und Cypridiniden findet, habe ich nichts entdecken können. Ich glaube danach, dass jede Kalkablagerung fehlt, ein Schluss, der durch das, was ich eben über das Zusammendrücken der Schale sagte, bestätigt wird.

Die Schale ist glatt, entbehrt jeder Skulptur; Haare fehlen so gut wie ganz, nur in der Rostralincisur und in ihrer Nachbarschaft findet sich am Rand ein dichte Reihe kurzer, starker Borsten (Tafel 1, Fig. 8). Das einzige wohl erhaltene Individuum zeigte einen grünlichen Schimmer.

Die innere Schalenlamelle ist noch viel zarter als die äussere, entfernt sich weit von ihr (vergl. Tafel 1, Fig 1 *i. L.*, *a. L.*), so dass der Schalenraum einen sehr umfangreichen Blutraum darstellt. Beide Schalenlamellen sind durch sehr zahlreiche feine Bindegewebsfasern mit einander verbunden, welche die Schale fein und dicht radiär (nach dem Centrum des Körpers hin) gestreift erscheinen lassen (siehe Fig. 1). Der Verlauf des Innenrandes (*Ir.*) in der Nachbarschaft der Rostralincisur ist aus Tafel 1, Fig. 8 ersichtlich. Am Ventralrand zieht er dem Schalenrand parallel, in geringer Entfernung von demselben. Zu einer deutlichen Verschmelzung beider Lamellen kommt es nicht. Der Saum ist schmal, ganzrandig oder fein gesägt, gestreift. In der hinteren Hälfte des Schlitzes zieht eine verstärkte Rippe dem eigentlichen Schalenrand parallel, nach innen von demselben und in seiner nächsten Nähe (Tafel 1, Fig. 7, 9 R_1). Kleine einzellige Drüsen sind über die ganze Schale zerstreut; etwas reichlicher entwickelt sind sie am Schalenrand; die Nerven der Schale sind viel schwächer entwickelt als bei den anderen Cypridiniden.

Bei der Untersuchung des eigentlichen Körpers thun wir gut, uns über die Lage der Organe zu orientiren, ohne das Thier aus der Schale herauszulösen, und das geht einigermaassen, da die Schale ziemlich durchsichtig ist. Wir vermögen sehr wohl Herz (*C.*), Frontalorgan (*Fr.*), Schliessmuskel (*Ml.*), Darm (*D.*), Furcalfeld (*Ff.*), 2. Antenne (*An.* 2), Putzfuss (*Pf.*), sowie den Rücken des Thieres mit seinen Muskeln zu erkennen (Tafel 1, Fig. 1, 2). Die nicht genannten Gliedmaassen liegen als unentwirrbare Masse auf den kleinen Raum zwischen 2 Antenne und vorderer Grenze des Furcalfeldes zusammengedrängt. Wie aus der Figur 1 ersichtlich, überwiegt die Rückenpartie des Körpers sehr stark, diese grössere hintere Hälfte wird nur zum kleinsten Theil erfüllt von dem Magen und den Geschlechtsorganen, der Rest stellt einen ausserordentlich umfangreichen Blutraum dar. Bei dem gezeichneten Thier, wie bei allen untersuchten der Art, war der Magen leer und stark contrahirt (vergl. unten), bei gefülltem Magen wird sich das Verhältniss etwas zu Ungunsten des Blutraumes verschieben, immerhin zeigt sich das, was schon bei Besprechung der Schale erwähnt wurde, doch noch viel auffälliger als dort: Die Bluträume haben einen ausserordentlichen Umfang erlangt, ihnen gegenüber tritt die feste Substanz des Körpers sehr zurück. Es ist mir aus der Gruppe der Krebse kein Beispiel für ein ähnliches Ueberwiegen der Bluträume bekannt geworden. Versuchen wir das Thier aus der Schale auszulösen, so erfolgt beim Anschneiden der Schale ein starker Ausfluss der Körperflüssigkeit, beide Schalenlamellen legen sich dicht aneinander, der Körper fällt zusammen; augenscheinlich genügt beim Fangen eine geringe Verletzung des Thieres, um das Blut ausfliessen zu lassen und den Körper arg zu entstellen.

Gliedmaassen. — Allgemein lässt sich von der Mehrzahl der Gliedmaassen sagen, dass sie verhältnissmässig kürzer sind als bei anderen Cypridiniden (vergl. Tafel 3): Die 1. *Antenne* (Tafel 3, Fig. 2) ist gestreckt und ziemlich schlank, sie ist 7 gliedrig, das 5. Glied trägt die gefiederte Sinnesborste der Cypridiniden; dieselbe ist in beiden Geschlechtern gleich stark entwickelt, die Fiederung ist nicht übrig reich. Das letzte Glied, an dem sich Reste einer Verschmelzung aus 7 und 8 nicht nachweisen lassen, trägt drei lange, spärlich gefiederte, starke Tastborsten, die beim ♀ etwa $\frac{4}{3}$, beim ♂ über 2 mal so lang als die Glieder 2–7, 2 kürzere Sinnesborsten, 1 kürzere starke und eine sehr kurze schwache Borste. Beim ♂ (Tafel 1, Fig. 24) tragen einzelne Borsten ähnliche Zweige mit saugnapfartigen Gebilden wie bei *Cypridina mediterranea;* jeder Zweig trägt nur einen kleinen Napf nahe der Basis, ist übrigens einfach. Solcher Zweige trägt die eine starke Tastborste 2, die kürzere 5. Die 2. *Antenne* (Tafel 3, Fig. 1) erinnert im Bau, wie die 1., lebhaft an die Gattung *Cypridina,* die Borste des 2. Gliedes des Schwimm oder Aussenastes ist kurz, ungefiedert, unbedornt, die übrigen Borsten sind sehr lang, gefiedert, nicht bedornt; der Dorn, der bei *Cypridina* neben der Basis der Borsten entspringt, fehlt. Der Innenast ist beim ♀ gestreckt, deutlich dreigliedrig, trägt am Ende eine lange Borste (Taf. 1, Fig. 21), beim ♂ ist er als Greiforgan entwickelt, das 3. Glied gegen das 2. einschlagbar (Taf. 1, Fig. 18, 19).

Die *Mandibel* (Tafel 3, Fig. 6) ähnlich wie bei *Cypridina,* besonders die Kauplatte (Taf. 1, Fig. 20) von ähnlicher Gestalt, die Borsten am Vorderrand des 3. Tastergliedes stark vermehrt, die Drüse erstreckt sich distal bis in die Basis des 3., proximal nur bis in die Mitte des 1. Tastergliedes. Bemerkenswerth ist ein dichter Besatz mit feinen Haarborsten, welche in Gruppen von 3–8 beisammen stehen; solche Gruppen finden sich am 1. Tasterglied, derber am Kaufortsatz (Tafel 1, Fig. 17, 20). 1. *Maxille* (Tafel 3, Fig. 3) von ähnlichem Bau wie bei *Cypridina,* ebenso die 2. *Maxille* (1. thoracale Gliedmaasse, Tafel 3, Fig. 4 ; Tafel 2, Fig. 8, 9). Die Borsten des letzten Gliedes sind stark vermehrt, die zahnartigen Gebilde der einen Reihe sind viel kräftiger als die der anderen, die der kräftigeren Reihe sind stark gekrümmt. Die 2. *thoracale Gliedmaasse* (Taf. 3, Fig. 7) ähnlich wie bei *Cypridina,* doch auffallend klein, mit ähnlichen Gruppen von Haarborsten wie der Mandibulartaster. *Putzfuss* (Tafel 3, Fig. 5) ausserordentlich lang und wohl entwickelt, die Ringe sehr kurz, das letzte $\frac{1}{7}$ mit sehr zahlreichen Borsten (ich schätze jederseits über 200), welche sämmtlich von typischen Bau; das Ende mit 1 langen Reihe sehr zahlreicher kleiner Zähne, welcher Reihe nur ein einzelner kleiner Zahn gegenüber steht (Tafel 1, Fig. 15). *Furca* mit 11 Dornenpaaren (die Zahl ist anscheinend constant). Die Dornen nehmen ziemlich regelmässig von der Spitze nach der Basis zu an Grösse ab; das in Figur 15, Tafel 2 gezeichnete Zurückbleiben des 5. Dornes findet sich nicht constant.

Die *Oberlippe* (Tafel 1, Fig. 3, *Ol.*) besteht aus einem unpaaren, nach vorn gerichteten Kamm und einem breiten, queren Wulst, welcher direct vor dem Munde liegt; die Mündungen der Drüsen finden sich am vorderen Rand des Kammes, sowie an den Seiten des queren Wulstes. Bemerkenswerth ist der Bau des *Magens* (Tafel 1, Fig. 10). Derselbe besteht aus einer inneren Epithelschicht; die Epithelzellen waren stets sehr dünn und hoch, was unzweifelhaft eine Folge der starken Contraction war, dann folgt ein structurlose, stark gefaltete Membran, dieselbe stammt vermuthlich von den Epithelzellen. Nach aussen von ihr liegen zahlreiche Muskelfasern (*Ml.*), die sich annähernd unter rechtem Winkel kreuzen, ein dichtes Netzwerk bilden, es folgt eine Schicht von Zellen, die dicht mit braunschwarzem Pigment erfüllt sind (Leberzellen), schliesslich ein dichtes Netzwerk von Bindegewebszellen, deren Ausläufer bis zum Rücken, in der Herzgegend bis zur Schale reichen; sie lassen den Magen wie mit einem dichten Pelz bekleidet erscheinen. Stets fand ich den Magen stark contrahirt und leer, augenscheinlich erfolgt im Netz oder bei der Conservirung stets eine starke Contraction und Entleerung. Bei der anderen Art, wo der Magenwand die Muskelfasern fehlen, fand ich im Magen Reste einer Salpe.

Sinnesorgane. — Paarige Augen. Dieselben erheben sich als kleine Warzen ziemlich frei über der Basis der 1. Antenne, hinter den Seitentheilen des Frontalorgans (Tafel 1, Fig. 3, *Oc.*). In jedem Auge finden sich 4 längliche, etwa birnförmige Blasen (die Zahl 4 scheint constant zu sein). Die Oberfläche der Blase ist glatt, auf der inneren Fläche der Wandung finden sich zahlreiche Rippen oder Leisten von stärkerem Lichtbrechungsvermögen, dieselben bilden ein Gewirr von ziemlich scharf begrenzten Linien, die einen unregelmässigen, meist geschlängelten Verlauf haben (Tafel 1, Fig. 14). Bei jüngeren Thieren ist die Anordnung der Rippen einfacher, auch zeigen sie bestimmte Beziehungen zu den zahlreichen Kernen, die sie meist im Bogen umziehen (Tafel 1, Fig. 11, da die Fig. 11 von *Gigantocypris pellucida* stammt, so ist es auch möglich, dass es sich um Artunterschiede handelt). Am proximalen, spitzen Ende geht die Blase in einen feinfasrigen Fortsatz über, den Nerven. Ich betrachte diese Blasen als eigenthümlich umgestaltete Rhabdome. Welche Function freilich diese Blasen haben, das ist schwer einzusehen. Ihre Verbindung mit einem Nerven spricht für ein Sinnesorgan, und hier scheint jede andere Deutung als die eines Sehorganes ausgeschlossen, ich komme auf die Frage noch einmal kurz zurück. Linsen habe ich in dem Auge vollständig vermisst, auch Pigment fehlte, doch kann es in Folge der Conservirung verloren gegangen sein; bei *G. pellucida* hatte die Blase ein bräunliche Färbung.

Das *Frontalorgan* ragt als grosser, nasenförmiger Körper oberhalb des

Ursprungs der 1 Antenne vor, im Profil zum Theil verdeckt durch den Stamm der 2 Antenne. Wir unterscheiden an demselben 3 in der Nase selbst liegende und 2 nahe der Nasenwurzel liegende Körper von dunklerer Färbung. Die Lage dieser Körper wird durch Fig. 3, 5, Tafel 1 veranschaulicht. Die in der Nase selbst liegende Gruppe besteht aus zwei grösseren dünnen, etwa senkrechten, nach unten divergirenden Platten (a) von annähernd dreieckiger Gestalt und einer kleineren, dünneren, länglichen Platte (b), welche sich zwischen die horizontalen Ränder der verticalen Platten schiebt. Die Platten bestehen aus einer annähernd homogenen Grundsubstanz, in der sich zahlreiche verzweigte Streifen einer dichteren, stärker färbbaren Substanz finden (Tafel 1, Fig. 12). Die Streifen sind nicht scharf gegen die Grundsubstanz abgegrenzt. Die Anordnung der Streifen lässt stellenweis ähnliche Beziehungen zu den Kernen erkennen wie beim Rhabdom (Tafel 1, Fig. 11).

Bei der horizontalen kleinen Platte, von der die Figur 12 stammt, ist das Bild ein ziemlich übersichtliches, in Folge der geringen Dicke der Platte liegen alle Streifen annähernd in einer Ebene, complicirter wird das Bild an den dickeren, verticalen Platten, bei denen die Zahl der dichteren Streifen eine grössere; bei den seitlichen Körpern (Fig. 3, 5 c), die einen etwa ovalen Querschnitt besitzen, ist sie naturgemäss am grössten; die Oberfläche derselben bietet das Bild einer sehr dichten, welligen Querstreifung (Tafel 1, Fig. 13). Auf Schnitten (Tafel 1, Fig. 4) zeigt sie sich in ganzem Umfang durchsetzt von dichteren, mit einander anastomosirenden Streifen. Die Innervirung erfolgt bei den seitlichen Körpern von der distalen Fläche aus, bei den senkrechten Lamellen anscheinend von der äusseren, bei der kleinen verticalen von der unteren Fläche, doch habe ich bei den zuletztgenannten keine volle Klarheit erlangt.* Die Kerne liegen stets lediglich an derjenigen Fläche, oder nahe derjenigen Fläche, an welcher die Nerven an den Körper herantreten. Die beiden seitlichen Körper sind von einem bräunlichen Pigment umhüllt, das einer sehr dünnen, structurlosen Membran aufliegt. Die Grenzen der Pigmentirung habe ich nicht sicher feststellen können. Der mittleren Gruppe scheint eine pigmentirte Hülle zu fehlen, doch dürfte es sich auch hier um einen Verlust des Pigmentes in Folge der Conservirung handeln.

Was die morphologische Deutung des Organs anbetrifft, so habe ich schon ausgesprochen, dass ich es als das Frontalorgan anspreche, und zwar halte ich die mittlere Gruppe für homolog dem unteren Abschnitt anderer Cypridiniden, die seitlichen Körper den oberen paarigen Abschnitten. An Stelle der Gruppen von Sehstäbchen sind solide Körper von ähnlicher Zusammensetzung getreten. Bei den seitlichen Körpern (c) erkennt man ohne Weiteres die Ubereinstimmung in der Art der Innervirung und Lage der Kerne. Wie bei den übrigen Cypridiniden haben wir dichtere und stärker lichtbrechende Stäbchen, welche einer homogenen Masse eingebettet sind, an der man häufig Zellgrenzen nicht zu erkennen vermag. Der wesentliche Unterschied würde in der Beschaffenheit der Stäbchen liegen, die hier viel länger und wellig gebogen sind, sich verzweigen, mit einander anastomosiren. Weniger klar liegen die Verhältnisse bei der mittleren Gruppe. Wir müssen annehmen, dass der ursprünglich einfache Abschnitt zunächst in drei kleinere Körper zerfallen ist. Bei der flächenhaften Entwicklung dieser Körper mussten die Sehstäbchen ihre Lage ändern, sie entwickelten sich parallel der Oberfläche der Körper. Der zitzen oder stabförmige Fortsatz des Frontalorgans fehlt; nur eine seinem Basalstück entsprechende Anhäufung von Kernen am unteren Rand der Nase und an der Nasenspitze ist als Rest desselben aufzufassen.

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* Ich muss die Unbestimmtheit der Resultate mit dem geringen Material, auf das ich angewiesen war, entschuldigen. Nur das eine wohl erhaltene ♂ lieferte für die Untersuchung des Frontalorgans brauchbare Resultate.

Das *Herz* ist auffallend zart und dünnhäutig, so dass es bei der Präparation des Thieres vollständig zusammenfällt. Der ausserordentlichen Vermehrung der Leibeshöhlenflüssigkeit entspricht also nicht eine Verstärkung des Circulationsapparates, im Gegentheil; ich vermuthe dass das Herz kaum noch functionirt. Die männlichen *Geschlechtsorgane* schliessen sich im Bau eng denen anderer Cypridiniden an, die weiblichen Geschlechtsorgane konnte ich nicht genauer untersuchen, da sämmtliche ♀ stark verdrückt waren; die Eier werden in grosser Zahl im Brutraum getragen. Der *Schliessmuskel* ist sehr schwach im Verhältniss zur Grösse des Thieres, die Zahl der Muskelbündel ist gering, die Bündel selbst sind schwach, doch zeigen sie deutliche Querstreifung.

Versuchen wir aus der Organisation des Thieres uns eine Vorstellung seiner Lebensweise zu bilden! Schon Eingangs sprach ich die Vermuthung aus, dass die Art ausschliesslich frei schwimmend lebt, sich nicht am Grunde bewegt. Für die Mehrzahl der Cypridiniden lehrt die Beobachtung des lebenden Thieres, dass dieselben vorwiegend an den Grund gebunden sind, aber auch für die Halocypriden, die man gewöhnlich als rein pelagische Formen betrachtet, habe ich die Ansicht ausgesprochen, dass sie sich wenigstens zeitweis am Grund aufhalten. Die Gründe, die ich für diese Ansicht an anderem Ort (Monographie der Ostracoden, p. 13) geltend machte, waren im wesentlichen folgende: 1. das hohe specifische Gewicht, besonders bedingt durch die Verkalkung der Schale; 2. die Körperform, der Mangel flächenhafter Ausbreitung, die ein freies Schweben ganz ohne oder mit nur geringem Kraftaufwand nicht gestattet; 3. der Bau der Gliedmaassen, welche zum Theil zum Anklammern geeignet sind. Wie verhält sich in dieser Beziehung *Gigantocypris?* Wie gesagt, fehlt der Schale jede Spur von Kalkablagerung, zudem tritt die feste Substanz der Leibeshöhlenflüssigkeit gegenüber sehr zurück, das specifische Gewicht kann kaum höher als das des umgebenden Mediums sein. Flächenhafte Ausbreitungen fehlen so gut wie bei anderen Ostracoden, doch ist, wie gesagt, der Umfang im Verhältniss zur Körpermasse ein sehr grosser, so dass man sich sehr wohl denken kann, dass das Thier ohne Zuhülfenahme seiner Ruder wie eine leichte Blase durch das Wasser treibt, ohne unterzusinken.

Von besonderem Interesse ist eine Betrachtung der Gliedmaasen. Die Gliedmaassen aller Cypridiniden sind ungeeignet zum Kriechen und Anklammern, und das gilt auch von *Gigantocypris,* wohl aber vermögen die Mehrzahl der Cypridiniden sich in den Grund einzugraben, die Gliedmaassen zeigen mancherlei Anpassung an diese Lebensweise, welche Anpassungen allerdings gerade bei der *Gigantocypris* am nächsten verwandten Gattung *Cypridina* am wenigstens ausgebildet sind. Wie verhält sich in dieser Beziehung *Gigantocypris?* Die 1. Antenne betheiligt sich bei *Cypridina* am Eingraben, bei *G.* ist sie viel schlanker, zum Eingraben weniger geeignet, besondere als Anpassungen an das Eingraben aufzufassende Eigenthümlichkeiten fehlen beiden Gattungen. 2. Antenne: Die Borste des 2. Schwimmastgliedes ist bei *Cypridina* kurz, ungefiedert, bedornt, bei *Gigantocypris* ebenfalls kurz, ungefiedert, aber unbedornt. Der Dorn, der bei *Cypridina* neben jeder Schwimmborste entspringt, und vermuthlich ebenfalls dem Eingraben dient, fehlt. Der Mandibulartaster betheiligt sich ebenfalls am Eingraben, er ist bei *Gigantocypris,* vergleichen mit der Maxille, verhältnissmässig kürzer als bei *Cypridina,* übrigens wohl entwickelt, was sich aus seinem Antheil an der Nahrungsaufnahme erklärt. Eine hervorragende Rolle spielt die Furca beim Eingraben, sie ist bei *Gigantocypris* auffallend kurz und schwach entwickelt, besonders sind die Dornen dünn und zerbrechlich. Wird die Furca nicht mehr zum Eingraben verwendet, so erscheint eine Gliedmasse, bestimmt die Furca abzubürsten ziemlich überflüssig, und in der That ist die 2. thoracale Gliedmaasse (Tafel 3, Fig. 7) ziemlich schwach entwickelt, während der Putzfuss eine ausserordentliche Länge erreicht, entsprechend der grossen Fläche, die er zu reinigen hat.

Uebrigens bedarf es kaum dieser Thatsachen, um den Beweis zu liefern, dass sich *Gigantocypris* nicht einzugraben vermag; der Umfang und die Zartheit der Schale allein würden eine ähnliche Bewegung unmöglich machen. Ich glaube nach allem Gesagten, dass sich *Gigantocypris* ausschliesslich frei schwimmend bewegt, nach meiner Auffassung würde es der einzige bis jetzt bekannt gewordene Ostracode mit dieser Lebensweise sein.

Mit der Frage nach der Art der Bewegung berührt sich eng eine andere, die, nach der Möglichkeit, die Schale zu öffnen. Nach der Darstellung, die ich oben vom Bau der Schale gegeben habe, drängt sich die Vorstellung auf, dass das Thier die Schale gar nicht zu öffnen vermag, das würde schon daraus folgen, dass beide Schalenhälften für ⅜ ihrer Peripherie fest mit einander verbunden sind. Auch die geringe Entwicklung des Schliessmuskels scheint diese Annahme zu befürworten. Man könnte sich wohl vorstellen, dass nur die 1. und 2. Antenne durch die Rostralincisur herausgestreckt werden könnten, dass sich, abgesehen vom Umherschwimmen, die Thätigkeit des Thieres darauf beschränkte, durch die Bewegung der 1. thoracalen Gliedmaasse (2. Maxille) einen Wasserstrom zu erzeugen, der bei geschlossener Schale durch die Rostralincisur ein, durch die hintere Oeffnung austräte und sowohl die Athmung vermitteln als auch die Nahrung zuführen würde. Im allgemeinen mag sich in der That das Leben der Thiere in dieser Weise abspielen, doch sprechen einige Gründe dafür, dass doch ein gewisses Oeffnen der Schale erfolgen kann; so wäre es unmöglich, die Beute, die ich ziemlich unzerkleinert im Magen von *G. pellucida* fand, durch die Rostralincisur allein in den Schalenraum und zum Mund zu bringen; weiter wäre eine Begattung, ein Vorstrecken des Penis ohne Oeffnen der Schale unmöglich. Unzweifelhaft ist aber der Mechanismus beim Oeffnen der Schale ein wesentlich anderer, es können nicht beide Schalenhälften als Ganzes von einander entfernt, sondern es können nur die Ränder auseinandergebogen werden, während die übrige Schale nur zum kleineren Theil in Mitleidenschaft gezogen wird; das würde ja auch bei der Weichheit und Biegsamkeit der Schale sehr wohl möglich sein. Immerhin scheint ein solches Oeffnen den postoralen Gliedmaassen und der Furca nur wenig Spielraum zur Bewegung zu lassen.

Noch bleibt die Frage zu erörtern, in welcher Beziehung die sehr auffällige Umgestaltung der Sehorgane zur Lebensweise steht? Da die gesammelten Thiere z. Th. aus bedeutender Tiefe stammen, so liegt der Gedanke nah, dass wir in der eigenthümlichen Form eine Anpassung an das Leben in grosser Tiefe zu sehen haben; vielleicht sind diese merkwürdigen Augen befähigt, noch bei sehr schwachem Licht Differenzen in der Helligkeit wahrzunehmen. Ich lasse noch eine kurze Gattungsdiagnose, sowie eine Differentialdiagnose beider Arten folgen.

Gigantocypris, nov. gen.

Schale dünn und zart, nicht verkalkt, beide Schalenhälften nur undeutlich gegen einander abgegrenzt, für etwa $\frac{2}{3}$ der Peripherie mit einander verschmolzen, mit kleiner, aber deutlicher Rostralincisur. Körper stark aufgetrieben. 1. Antenne gestreckt, 7 gliedrig, Sinnesborste des 5. Gliedes schwach gefiedert, in beiden Geschlechtern gleich; beim ♂ 2 Borsten des letzten Gliedes mit Haftorganen; 2 Antenne: die erste Borste des Schwimmastes kurz, ungefiedert, unbedornt. Innenast dreigliedrig, beim ♂ als Greiforgan functionirend, das letzte Glied einschlagbar, Mandibel mit einfachem, einspitzigem, rückwärts gerichtetem Fortsatz des Basalgliedes und wohl entwickeltem 4 gliedrigem Taster, 1. Maxille von typischem Bau, mit 3 Kaufortsätzen und 3 gliedrigem Taster. 1. thoracale Gliedmaasse (2. Maxille) mit 2 Reihen zahnartiger Borsten. 2. thoracale Gliedmaasse deutlich 4 gliedrig, mit schwacher Musculatur. Putzfuss sehr lang, mit sehr zahlreichen Borsten und einer Reihe kleiner Zähne. Furca ohne deutliche Differenzirung in Haupt und Nebendornen. Oberlippe umfangreich, ohne zitzenartige Fortsätze, die Mehrzahl der Drüsen mündet auf einer Kante am Vorderrand. Frontalorgan sehr umfangreich, die oberen Gruppen weit von der mittleren getrennt, die mittlere in 3 Körper aufgelöst, ohne zitzen oder stabförmigen Fortsatz. Paarige Augen vorhanden (in beiden Geschlechtern ?), mit 4 eigenthümlich umgestalteten, blasenförmigen Elementen, ohne Linsen.

Wie schon hervorgehoben, schliesst sich *Gigantocypris* der Gattung *Cypridina* eng an, zeigt eine weitgehende Uebereinstimmung im Bau der Gliedmaassen. Als unterscheidende Merkmale wären hervorzuheben: der Bau der Schale, der Oberlippe und des Frontalorganes. Die oben gegebene Beschreibung bezog sich auf *Gigantocypris Agassizii*, neben dieser durch 5 Individuen (4 ♀, 1 ♂) vertretenen Art fand sich noch ein Individuum einer zweiten Art; obwohl nur ein noch nicht geschlechtsreifes ♂ vorlag, habe ich doch geglaubt, bei dem besonderen Interesse, welches sich an die Gattung knüpft, die Art beschreiben zu sollen, zumal scharfe unterscheidende Merkmale existiren.

Gigantocypris pellucida, n. sp.

Schale, Gliedmaassen und sonstiger Körperbau wie bei *Agassizii;* am Putzfuss war die Zahl der Zähne in der Reihe an der Spitze viel kleiner

(7 anstatt etwa 60; Tafel 1, Fig. 16, 15). Neben den typischen Borsten (Tafel 1, Fig. 23), existiren solche mit einfachem pinselartigem Ende (Fig. 22), letztere sind viel seltner als die erstgenannten. In der Magenwand fehlen die Muskelfasern, an ihrer Stelle finden sich nur dünne, anscheinend nicht contractile Fasern, entsprechend ist der Darm an conservirtem Material nicht contrahirt; ferner fehlen die zur Leibeswand verlaufenden Bindegewebsfasern. Das untersuchte Thier stand vor der zur Geschlechtsreife führenden Häutung, es mass 16 mm., danach dürfte die Art etwas kleiner sein als *Agassizii.*

Vorkommen: Sämmtliche Thiere sind gefischt nahe der Westküste von Central Amerika, nämlich:—

0° 36′ n. Br.	82° 45′ w. L.
0° 54′ n. Br.	91° 9′ w. L.
2° 34′ n. Br.	92° 6′ w. L.
26° 48′ n. Br.	110° 45′ w. L.
27° 3′ n. Br.	111° 0′ w. L.

in Tiefen von etwa 1700 Faden bis 100 Faden (offenes Netz).

Vermuthlich bezieht sich auch die folgende Angabe* auf eine Art der Gattung *Gigantocypris:* Der Challenger brachte zwischen den Prinz Edwards und Crozet Inseln aus einer Tiefe von 1375 oder 1600 Faden mit dem Schleppnetz einen Ostracoden herauf, dessen weiche, skulptirte Schale eine Länge von 25 mm. und eine Höhe von 16 mm. hatte. Der Deckel (?) allein ist 3 mm. lang. Wahrscheinlich gehört dieser Ostracode, von dessen Körper nur der Kopf erhalten ist, zu keiner der bis jetzt bekannten Familien. Die Angabe, dass nur der Kopf erhalten sei, dürfte sich vielleicht aus der geringen Grösse des Körpers, vergleichen mit der Schale, erklären. Leider fehlen nähere Angaben über das Thier, in den Challengerostracoden ist es nicht erwähnt.

HALOCYPRIDÆ.

Gesammelt sind 4 Arten *Conchœcia* (*Conchœcissa*) *armata* Cls., 1 ♀ gefangen unter 10° 14′ n. Br. 96° 28′ w. L. in 100 Faden Tiefe. Ferner je 1 ♀ von 2 unbeschriebenen Arten; da der Fund zu einer scharfen Characterisirung der Arten nicht genügt, unterlasse ich es, die Arten zu beschreiben. Schliesslich fand sich ein reichliches Material einer sehr stattlichen *Conchœcia:*

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* Briefe von der Challengerexpedition von R. v. Willemoes—Suhm. Zeitschrift wissensch. Zoologie, Bd. 24, p. XIII.

Conchœcia Agassizii, n. sp.

(Tafel 2, Fig. 1-7, 12-14, 16-18.)

Schale sehr derb, ungewöhnlich derb und widerstandsfähig für einen Halocypriden, besonders für eine *Conchœcia*, indessen im Verhältniss zum Körper zu klein, so dass sie den Körper nicht ganz umhüllt, vielmehr stark klafft. Die des ♀ nicht ganz noch einmal so lang wie hoch (Höhe zur Länge etwa 1 : 1,9), am breitesten etwa auf ⅔ der Länge, von wo sich die Schale stark nach vorn verschmälert, hintere untere Ecke breit gerundet, der Hinterrand gerade, bildet mit dem Dorsalrand einen spitzen Winkel mit abgerundeter Spitze. Rechte und linke obere Ecke nicht deutlich verschieden. Schale des ♂ etwas gestreckter, etwas über noch ein mal so lang wie hoch, nach vorn weniger stark verschmälert; der Winkel, welchen Dorsal- und Hinterrand mit einander bilden, grösser als beim ♀, doch immer noch kleiner als ein rechter, der Rostralfortsatz des ♂ nur wenig stärker in die Höhe gebogen als beim ♀, mit kurzer, abgesetzter Spitze. In beiden Geschlechtern zeigt die Schale eine schwach entwickelte, nur schwer nachweisbare Skulptur; dieselbe besteht aus zahlreichen undeutlichen Linien, welche in der vorderen Hälfte eine parallele Streifung oder langgestreckte Rhomben, in der hinteren Hälfte eine polygonale Felderung bilden. Die Streifen verlaufen annähernd senkrecht zur Rückenlinie. Die unsymmetrischen Drüsengruppen sind wohl entwickelt, die Männchendrüsen habe ich nicht auffinden können.

1. Antenne des ♂: die Hauptborste sehr lang, über 3 mal so lang als die 1. Antenne, mit sehr zahlreichen rückwärts gerichteten Spitzen, ich zähle gegen 100 Paare; die Paare stehen sehr dicht und regelmässig neben einander, so dass die eine Spitze die benachbarte im Profil verdeckt, folgen sich sehr dicht, verändern proximalwärts Gestalt und Anordnung nicht oder nur unbedeutend; auf die rückwärts gerichteten Spitzen folgen distal noch einige schwächere, vorwärts gebogene borstenartige Anhänge (Tafel 2, Fig. 18). Die zwei schwächeren Borsten sind annähernd gleich lang, etwas länger als die halbe Hauptborste, sie tragen einzelne vorwärtsgerichtete Spitzen. Von den beiden Sinnesborsten erreicht die distale eine Länge von mehr als ⅔ der 1. Antenne, sie ist sonst in der Gattung *Conchœcia* durchweg viel kürzer. Beim ♀ ist die 1. Antenne viel schwächer als beim ♂, zeigt aber eine wohl entwickelte Muskulatur, die Hauptborste erreicht die 2–3fache Länge der 1. Antenne, die 4 gleichlangen Sinnesschläuche etwa die Länge der 2 ersten Glieder.

Nebenast der 2. Antenne des ♂ (Tafel 2, Fig. 3–5, 7): das Basalglied von typischer Form, die eine Borste dünn gefiedert, das 2. Glied trägt ausser den 2 starken endständigen Borsten gegenüber dem Ursprung des letzten Gliedes zwei schwache, schlanke Borsten, von den beiden endständigen Borsten erreicht die eine eine ausserordentliche Länge, ist etwa doppelt so lang wie der Aussenast mit seinen Schwimmborsten, die andere erreicht nicht ganz die halbe Länge der ersten. Am letzten hakenartig gebogenen Glied bleiben die 3 Borsten sehr kurz, erreichen etwa nur ⅛ der längsten Borste des Innenastes, der Haken ist auf beiden Seiten stark gebogen, in eine Spitze ausgezogen, rechts und links in der Gesammtform nicht sehr verschieden; auf der einen Seite zeigt er nahe der Basis 2 einander gegenüberstehende zahnartige Vorsprünge.

Beim ♀ (Tafel 2, Fig. 6, 13) ist der Nebenast kürzer, die längste Borste erreicht noch nicht ⅔ der Länge wie beim ♂, die Borsten des letzten Gliedes bleiben wie beim ♂ sehr kurz, an Stelle der 2 überzähligen Borsten des vorletzten Gliedes beim ♂ findet sich nur eine. Beide Borsten des 1. Gliedes sind ungefiedert.

Frontalorgan mit einfachem, erweitertem Endstück, dessen Form nicht besonders charakteristisch; das des ♀ dem des ♂ ähnlich, nicht ganz noch einmal so lang als die 1. Antenne, das erweiterte, nicht beweglich abgesetzte, stark bedornte Endstück schlanker als beim ♂.

Das Thier erreicht eine Grösse von 4,8 mm.

Gefischt im Golf von Californien in einer Tiefe von etwa 700 Meter (offenes Netz).

FIGURENERKLAERUNG.

a. L.	Aeussere Lamelle.	*N.*	Nerv.
An_1, An_2.	Erste, zweite Antenne	*Oc.*	paariges Auge.
C.	Herz.	*Oe.*	Œsophagus.
D.	Darm.	*Ol.*	Oberlippe.
Dr.	Drüsenmündung.	*Pe.*	Penis.
F.	Furca.	*Pf.*	Putzfuss.
Ff.	Furcalfeld.	*R.*	Rand.
Fr.	Frontalorgan.	*R_1.*	innere Randleiste.
G.	Gehirn.	*S.*	Saum.
i. L.	innere Lamelle.	*T.*	Hoden.
Ir.	Innenrand.	*Vl.*	Verwachsungslinie.
Ml.	Muskel.		

TAFEL 1.

Fig. 1–5. *Gigantocypris Agassizii.*

Fig. 1, 2. ♂, ganzes Thier, von der Seite und von unten. 3 ×

Fig. 3. Ventrale Körperhälfte nach Entfernung der Gliedmaasen. 7 ×. Die Buchstaben a, b, c, bezeichnen die gleichen Theile wie in Fig. 5.

Fig. 4. Schnitt durch einen seitlichen Körper (c) des Frontalorganes. 100 ×

Fig. 5. Frontalorgan von vorn. 7 × (a, b, c vergl. Fig. 3).

Fig. 6, 7. *Gigantocypris pellucida* ♂ juv. Rostralincisur und hinteres Ende des Schalenschlitzes von vorn, resp. unten. 7 ×

Fig. 8–10. *Gigantocypris Agassizii.*

Fig. 8, 9. ♂ Rostralincisur und hinteres Ende des Schlitzes von innen. 8 ×

Fig. 10. ♀ Schnitt durch die Magenwand. 162 ×

Fig. 11. *Gigantocypris pellucida.* Einzelnes Rhabdom. 100 ×

Fig. 12–15. *Gigantocypris Agassizii.*

Fig. 12. ♂ Distales Ende des Körpers b des Frontalorganes von der Fläche. 100 ×

Fig. 13. ♀ Körper c. des Frontalorganes. 30 ×

Fig. 14. ♂ Stück eines Rhabdoms. 162 ×

Fig. 15. ♀ Spitze des Putzfusses. 100 ×

Fig. 16. *Gigantocypris pellucida.* ♂ juv. Spitze des Putzfusses. 100 ×

Fig. 17–21. *Gigantocypris Agassizii.*

Fig. 17. ♀ Stück vom 1. Tasterglied der Mandibel. 66 ×

Fig. 18, 19. ♂ Spitze des Innenastes der 2. Antenne, 162 ×, und Innenast. 30 ×

Fig. 20. ♀ Kaufortsatz der Mandibel. 66 ×

Fig. 21. ♀ Innenast der 2. Antenne. 30 ×

Fig. 22, 23. *Gigantocypris pellucida.* ♂ juv. Spitzen von 2. Borsten des Putzfusses. 400 ×

Fig. 24. *Gigantocypris Agassizii.* ♂ 2 letzten Glieder der 1. Antenne mit einem Theil der Borsten (weggelassen sind die 2 Sinnesborsten und eine starke Borste des letzten Gliedes). 66 ×

TAFEL 2.

Fig. 1–7. *Conchœcia Agassizii.*

Fig. 1, 2. Schale im Profil 1 ♂, 2 ♀; 19 ×

Fig. 3. ♂ Innenast der 2. Antenne mit der Basis der Borsten, von innen, unter Deckglas. 100 ×

Fig. 4. ♂ 2 Letzten Glieder des rechten Innenastes. 100 ×

Fig. 5. ♂ Rechter Innenast. 26 ×

Fig. 6. ♀ Innenast, frei liegend, sonst wie 3. 100 ×

Fig. 7. ♂ Rechter Innenast, frei liegend, sonst wie 3. 100 ×

Fig. 8–10. *Gigantocypris Agassizii.*

Fig. 8, 9. Kautheil der 1. thoracalen Gliedmasse (2. Maxille) von innen, 26 × und Zähne beider Zahnreihen. 100 ×

Fig. 10. Erster Furcaldorn von aussen. 50 ×

Fig. 11. *Gigantocypris pellucida.* ♂ juv. Furca und Anlage des Penis von vorn. 7 ×

Fig. 12–14. *Conchœcia Agassizii.*

Fig. 12. ♀ Kaufortsatz der Mandibel von innen, am Taster haftend. 216 ×.

Fig. 13. ♀ Innenast der 2. Antenne. 26 ×

Fig. 14. ♀ 1. Antenne und Frontalorgan. 66 ×

Fig. 15. *Gigantocypris Agassizii* ♀ Furca (nur ein Ast ist gezeichnet). 19 ×

Fig. 16–18. *Conchœcia Agassizii* ♂.

Fig. 16. 1. Antenne und Frontalorgan, rechte Antenne entfernt. 66 ×

Fig. 17, 18. Bezahntes Stück einer Nebenborste und Ende der Zahnreihe der Hauptborste der 1. Antenne, beides 400 ×

TAFEL 3.

Sämtliche Figuren stellen Gliedmaassen eines geschlechtsreifen ♀ von *Gigantocypris Agassizii* von 23 mm. Schalenlänge dar; alle Figuren 10 × vergrössert.

Fig. 1. 2. Antenne.

Fig. 2. 1. Antenne.

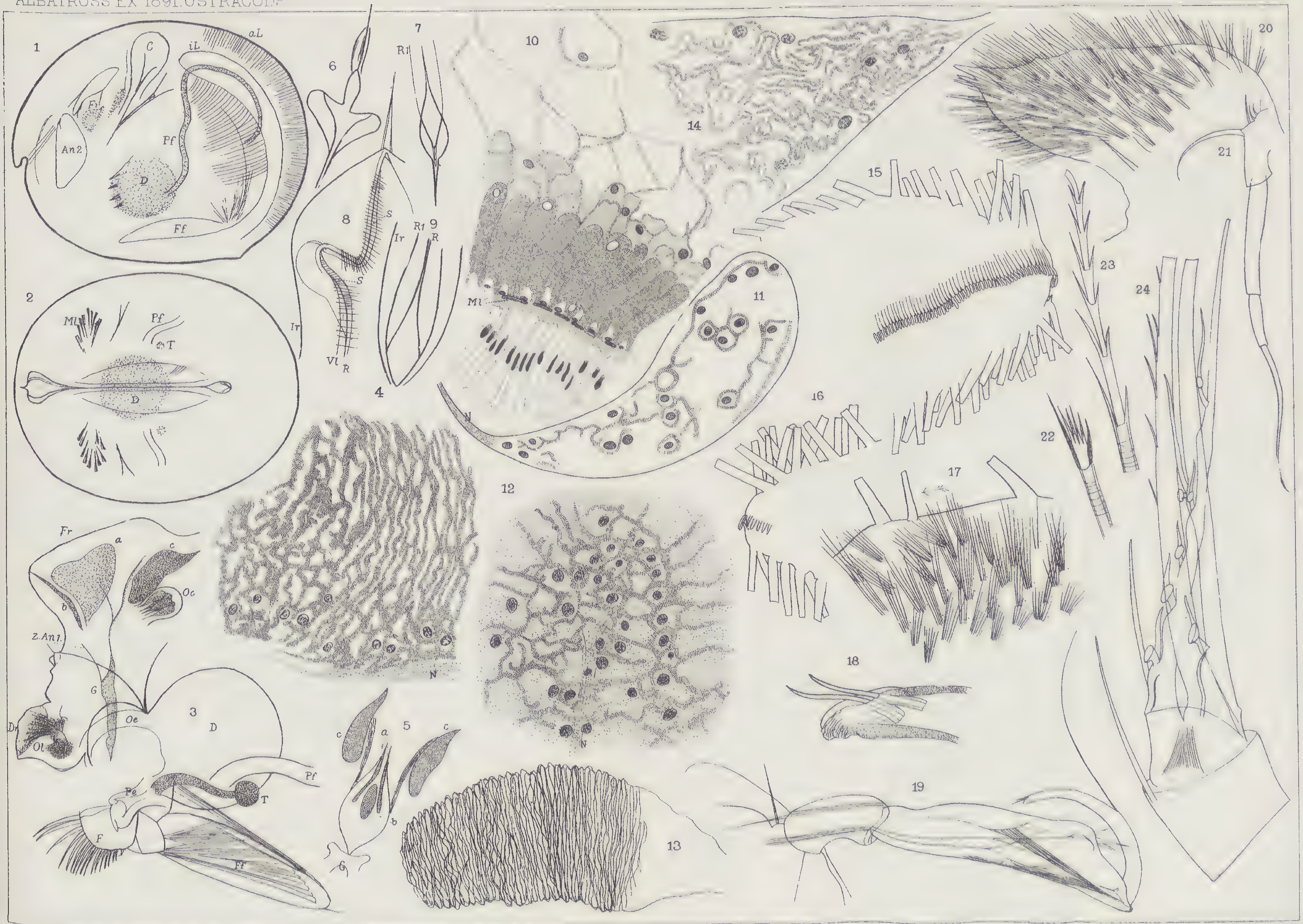
Fig. 3. 1. Maxille.

Fig. 4. 1. thoracale Gliedmaasse (2. Maxille).

Fig. 5. Putzfuss.

Fig. 6. Mandibel.

Fig. 7. 2. thoracale Gliedmaasse.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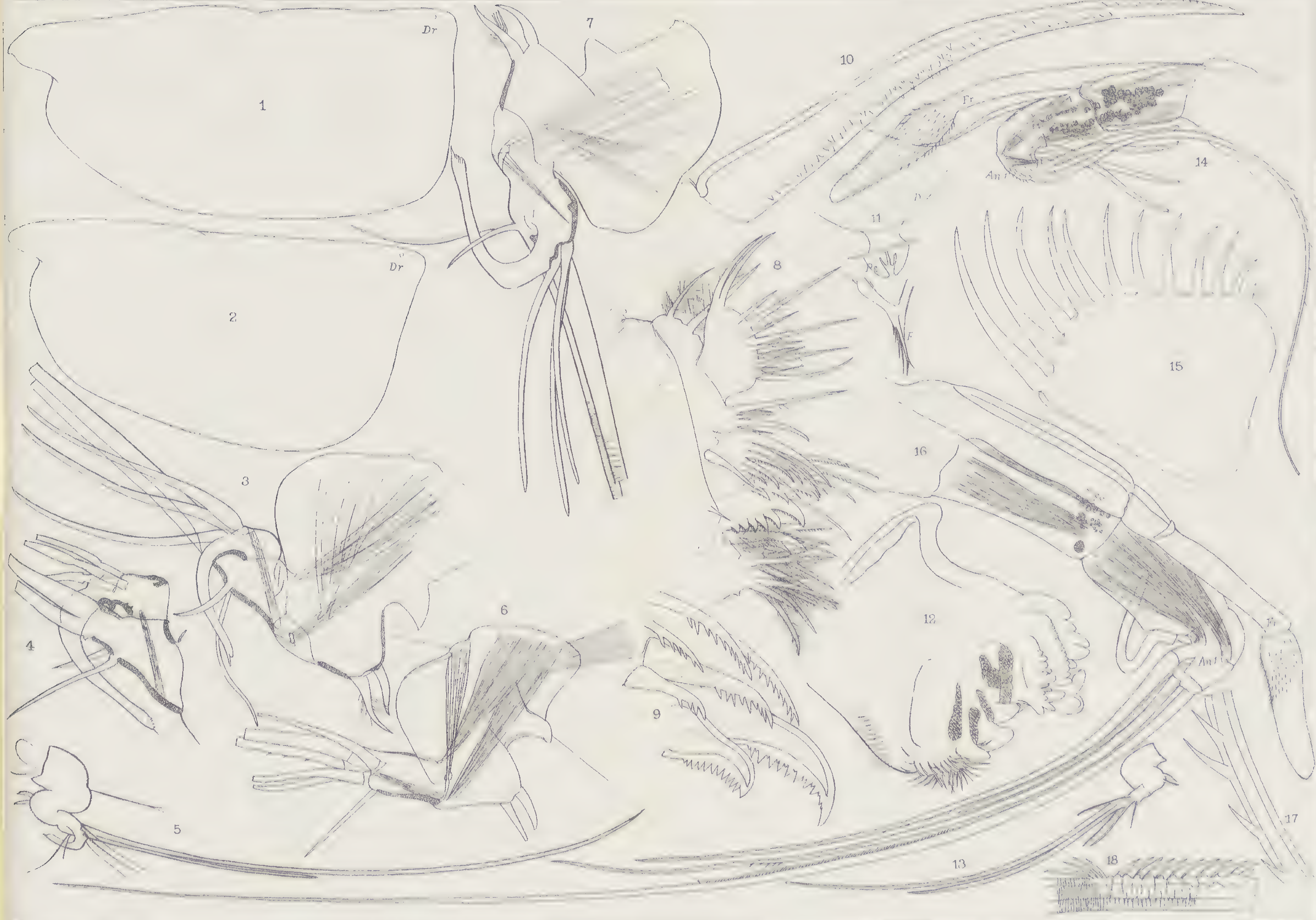
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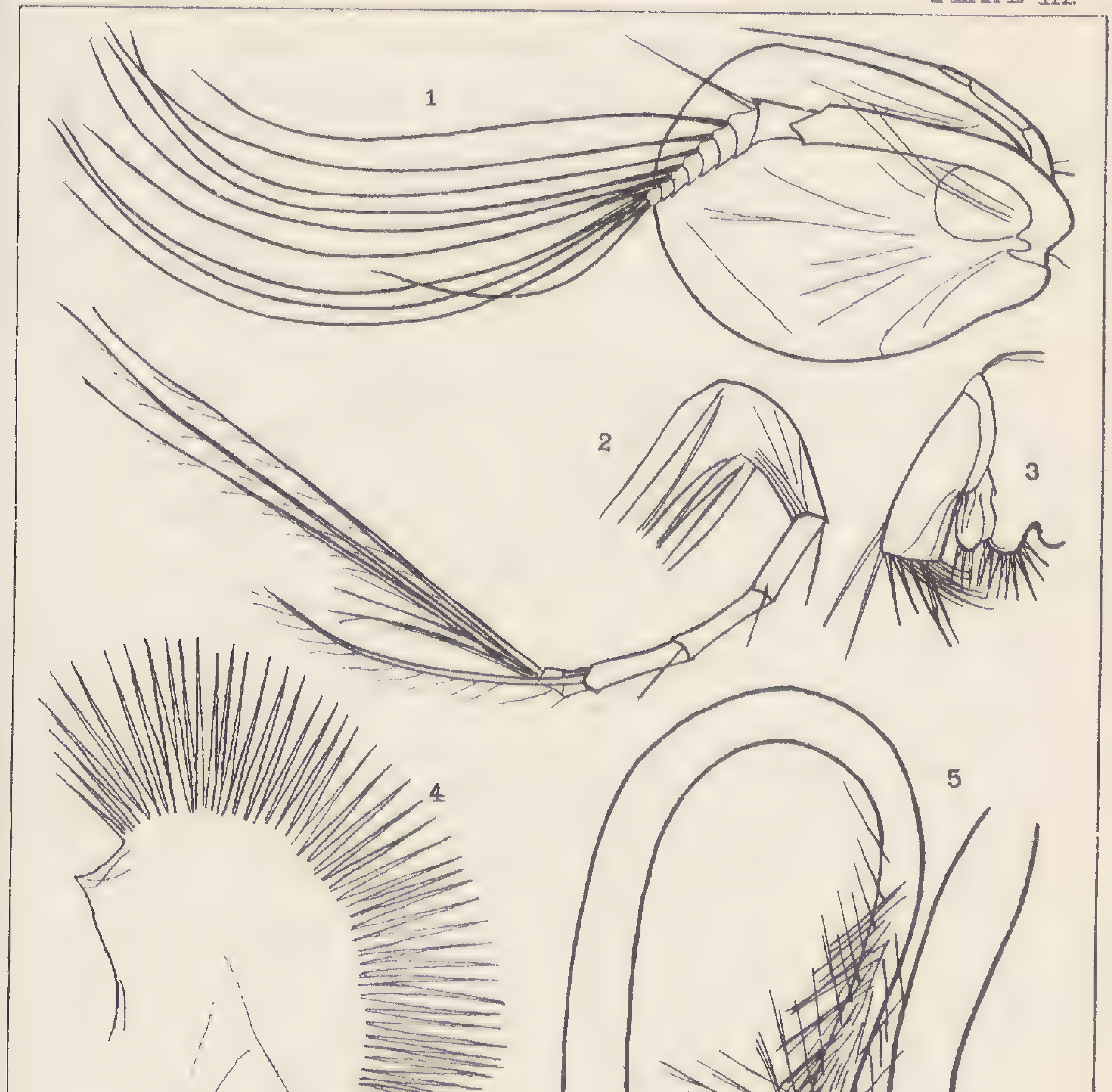
ALBATROSS EX.1891.OSTRACODS PLATE III.



G.H.Müller, del. E. Crisand, lith., New Haven, Ct.

Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
Vol. XXVII. No. 6.

STUDIES IN MORPHOGENESIS.

IV.

A PRELIMINARY CATALOGUE OF THE PROCESSES CONCERNED IN ONTOGENY.

By C. B. Davenport.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
November, 1895.

No. 6. — *Studies in Morphogenesis.* — IV. *A Preliminary Catalogue of the Processes concerned in Ontogeny.*[1] By C. B. Davenport.

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Most important perhaps of all the problems which the biologist sees lying unsolved before him is that of the development of the individual, — a problem to which, from the time of Aristotle, zoölogists have repeatedly turned, although scarcely hoping for its eventual solution.

Without attempting to consider the various theories of Ontomorphogenesis which have at different times been offered, it is sufficient to state that it is now generally agreed that ontogenesis is a process, or rather a complex of processes, taking place in the protoplasm of the developing individual.

Now it is a highly probable belief that no movement takes place in protoplasm except as a response to stimuli. The very fact that ontogenesis is a complex of actions indicates that there must be a large number of stimuli raining in upon the different parts of the developing protoplasm to which they respond.

In order to gain some idea of what the stimuli are, it is first necessary to analyze the ontogenetic complex of processes into its simple elementary ones.

It is the aim of this paper to make such an analysis into the elementary ontogenetic processes as a basis for determining the nature of the exciting stimuli.

---

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, E. L. Mark, Director, No. L.

Other authors have devoted chapters to "Developmental Processes," but none of these can be considered as at all complete.

Thus O. Hertwig, in his "Text-Book of the Embryology of Man and Mammals" (English Translation by E. L. Mark, 1892, p. 76), has a "General Discussion of the Principles of Development." He recognized two main ones: (1) the principle of unequal growth (producing folds which are either invaginations or evaginations, and which may unite along their edges); and (2) the principle of histological differentiation.

Minot, in his Human Embryology, in a chapter on "Differentiation," seems to think also that these two processes are sufficient to explain the differentiation of organs.

More important in this connection than either of the preceding is the recent paper of Herbst in the "Biologisches Centralblatt" (Vol. XIV. Nos. 18–22). This author, after reviewing the literature upom taxism and tropism, explains as phenomena of the same order certain ontogenetic processes. He has not, however, attempted to catalogue all the ontogenetic processes.

Before beginning the present catalogue, I may state that I distinguish between ontogenetic *principles* and ontogenetic *processes*. Under the first head I include such laws of development as terminal growth, repetition of parts and bilateral symmetry in development. These I have not attempted to catalogue. The present paper is concerned only with the latter group, which comprises the different elementary operations or actions exhibited in ontogeny.

These may be divided into two classes: 1. the grosser ontogenetic processes; and 2. histogenic processes. This paper deals with the first class only.

In discussing the grosser ontogenetic processes we may distinguish (A) those of a more general nature from (B) the more special ones.

A. The *general ontogenetic processes* comprise those of a general physiological character. Of these, at least three are commonly recognized: —

1. Growth (including both the results of assimilation and of imbibition of water).

2. Nuclear division.

3. Secretion.

B. The *special* ontogenetic processes are differential in character, i. e. the differentiation of the body is effected by them.

These processes may be classified, first of all, according to the form of the protoplasmic bodies in which they occur. These exist either as (I.) isolated cells, or as larger multinucleated bodies. Of the latter we may recognize three classes: (II.) bodies extended chiefly in one direction, — threads, fibres, tubules; (III.) those which extend as a layer; and (IV.) those in which the three dimensions are more nearly equal, forming solid masses.

I propose now to discuss the processes occurring in each of these four classes.[1]

I. Ontogenetic Processes occurring in Migratory Protoplasmic Bodies — Mesenchyme.[2]

1. *Migration of Nodal Thickenings in a Protoplasmic Mesh-work.* This process is found, for example, in many Arthropod eggs before the formation of the peripheral blastoderm. (Figure 1.) No one can doubt that protoplasm extends throughout the whole egg in the form of a mesh- or foam-work, whose interspaces are filled with yolk. The protoplasm is aggregated around the nuclei at certain nodal points, which later migrate to the surface or through the yolk as vitellophags. Cf. K. & H.,[3] Figs. 7, 363, 417, 448, 472, 473, 771.

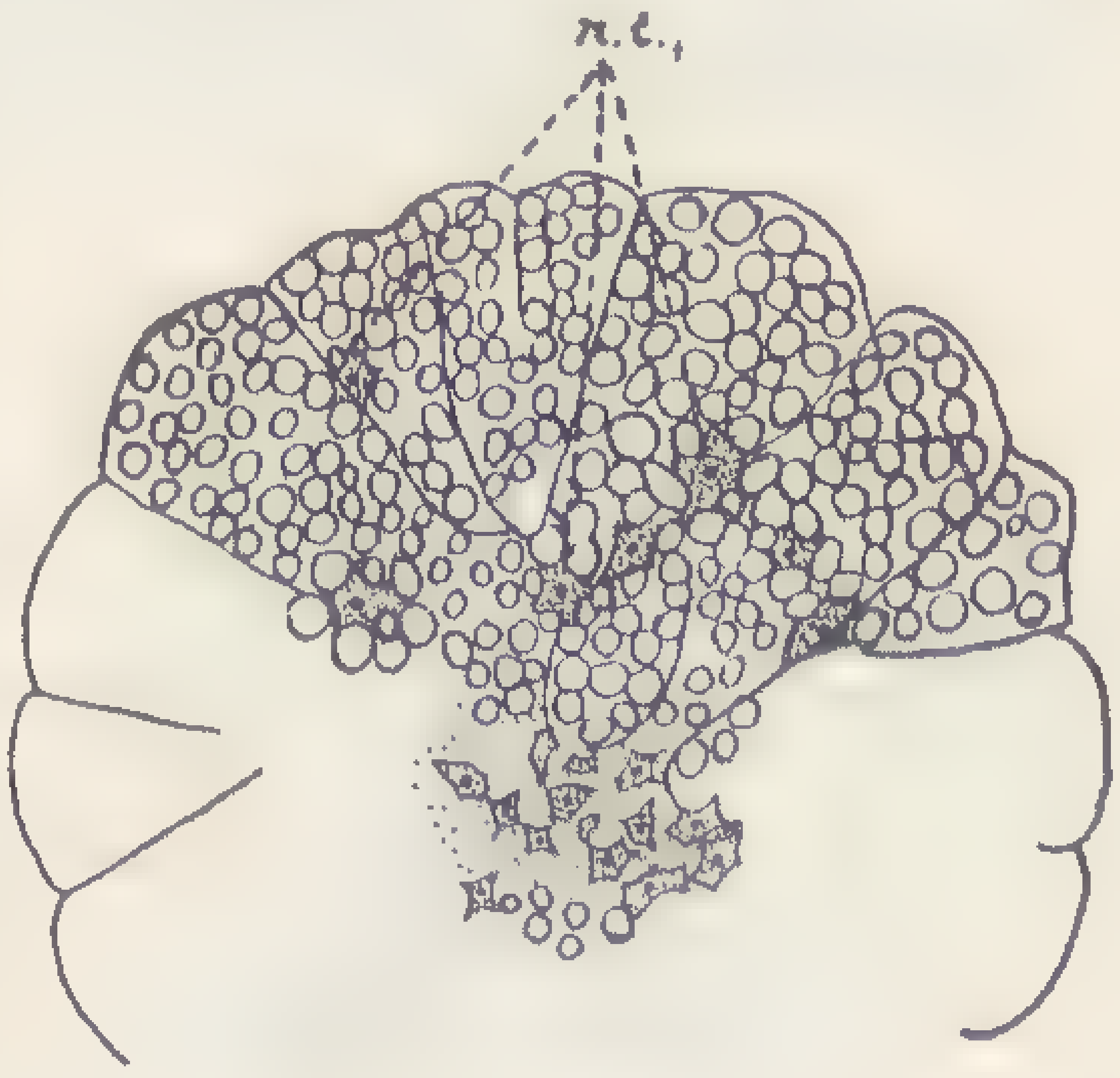

FIG. 1.

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[1] It may be a cause of dissatisfaction to some that this classification is not "strictly dichotomous"; it is still more serious that the different heads are not of co-ordinate rank or mutually exclusive. Of course, the classification employed in this list cannot be regarded as a final one. I hope, however, that I have succeeded in an attempt roughly to arrange the different items in a logical fashion.

[2] In the present paper the word "mesenchyme" is used as a name for all amœboid, migrating cells, of whatever origin.

[3] Throughout this paper certain abbreviations are used in referring to the books from which the figures are copied. These are: K. & H. for Korschelt und Heider's "Entwicklungsgeschichte"; M. for Minot's "Human Embryology"; and H.-M. for Hertwig's "Text-Book of Embryology of Man and Mammals," translated by Mark.

Fig. 1. Section through an egg of a Myriapod (Geophilus), showing the nodal thickenings (*n. t.*) in the act of migrating towards the periphery of the egg. See K. & H., Fig. 449.

2. *Free Migration of Amœboid Bodies.* This process differs from the preceding in that the migrating bodies are not connected together. It is characteristic of mesenchyme. I know that Dreyer ('92, Jena. Zeitschr., XXVI. 359) and Sedgwick ('94, Quart. Jour. Micr. Sci., XXXVII.) insist that the cells of mesenchyme, which are usually considered unconnected like so many amœbæ, are really nodal thickenings in an extensive mesh-work or foam-work, the intervening fluids being the, in some places confluent, vacuoles. Wherever mesenchyme has this structure, its migrations belong to the preceding class. But I believe there still remains a considerable residuum of cases falling under this head.

This process is capable of division into two subprocesses; viz. (*a*) migrating of mesenchyme out of a protoplasmic layer in order to become free, and (*b*) migrating through fluid-filled spaces. Both these processes are illustrated in Figure 2. Further illustrations will be found in K. & H., Figs. 102, 103, 170, 175–180, 182, 186, 188–190, 207, 285, 559, 596–598, 628, 698, 733, 809, and M., Figs. 121, 234, 239.

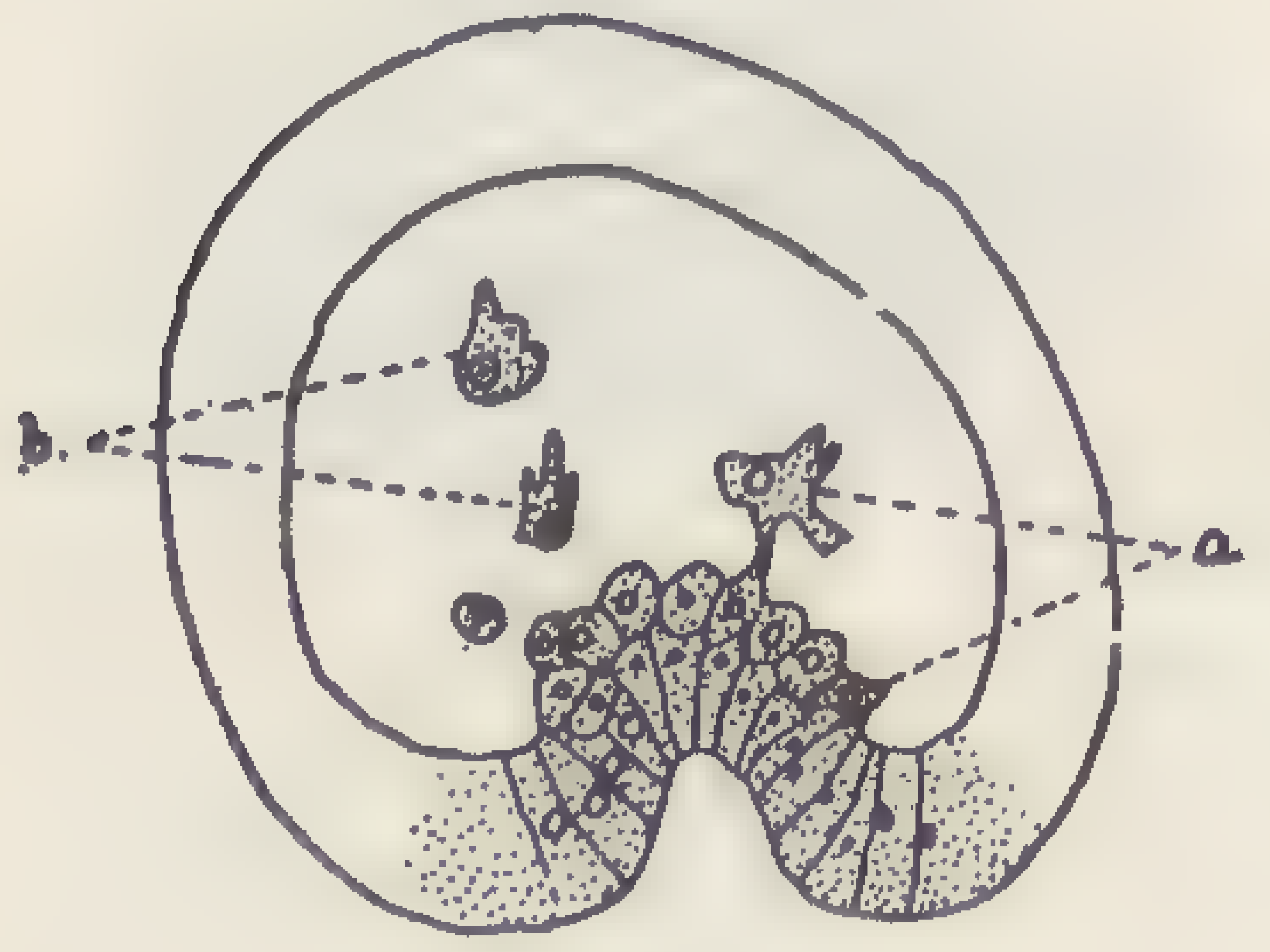

Fig. 2.

The migratory processes named below are of subordinate rank to Nos. 1 and 2. But, being fairly well marked and of considerable importance, it is convenient to treat them as co-ordinate.

We may distinguish, first, movements of mesenchymatous elements towards and from each other, and, secondly, movements with reference to other protoplasmic masses.

3. First among the former we recognize the *aggregation of migratory protoplasmic bodies*, and here we may distinguish three sub-classes according to the form of the resulting body.

a. First we have the *aggregation of mesenchyme into a body with a chiefly linear dimension*, — the formation of a thread, cord, or tubule.

Examples of this process in Invertebrates are seen in the formation of the kidney of Lamellibranchs, which seems to be laid down as a cord-like aggregation of mesenchyme, and in that of the thread of the yolk glands

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Fig. 2. Section of Holothurian larva showing mesenchyme migrating out of a layer at *a*, and through a fluid-filled space at *b*. From H.-M., Fig. 109.

of Turbellaria according to Iijima (Zeitschr. f. wiss. Zool., XL. 455). Among Vertebrates, we have the observations of Paterson, (Figure 3,) according to which the sympathetic nerve arises by the aggregation of mesenchymatous elements into a strand; of His, who affirms the origin of the spinal and the olfactory ganglia from migrating cells; and of various authors, who make blood capillaries and lymph vessels arise by this process (cf. M., pp. 217, 413).

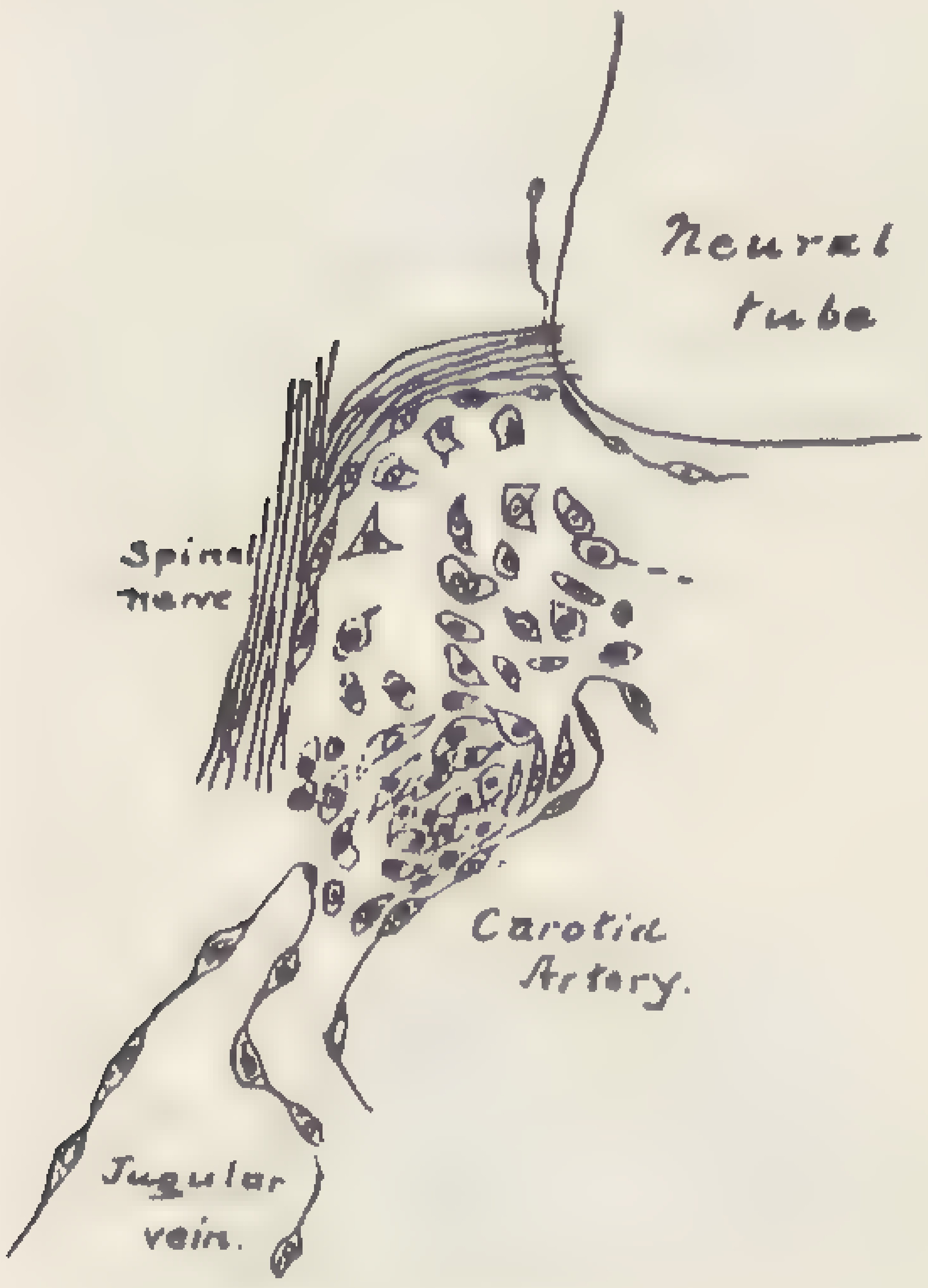

Fig. 3.

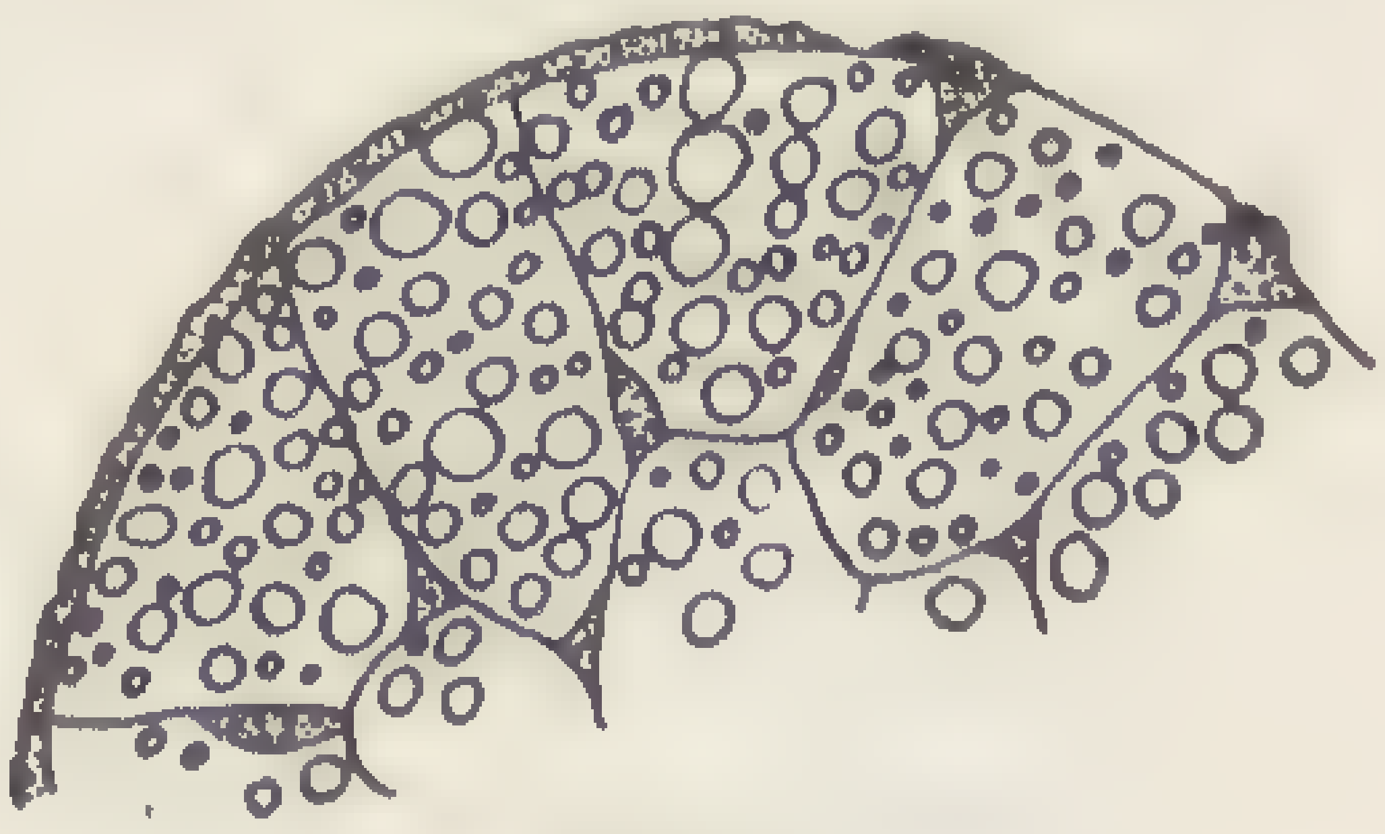

Fig. 4.

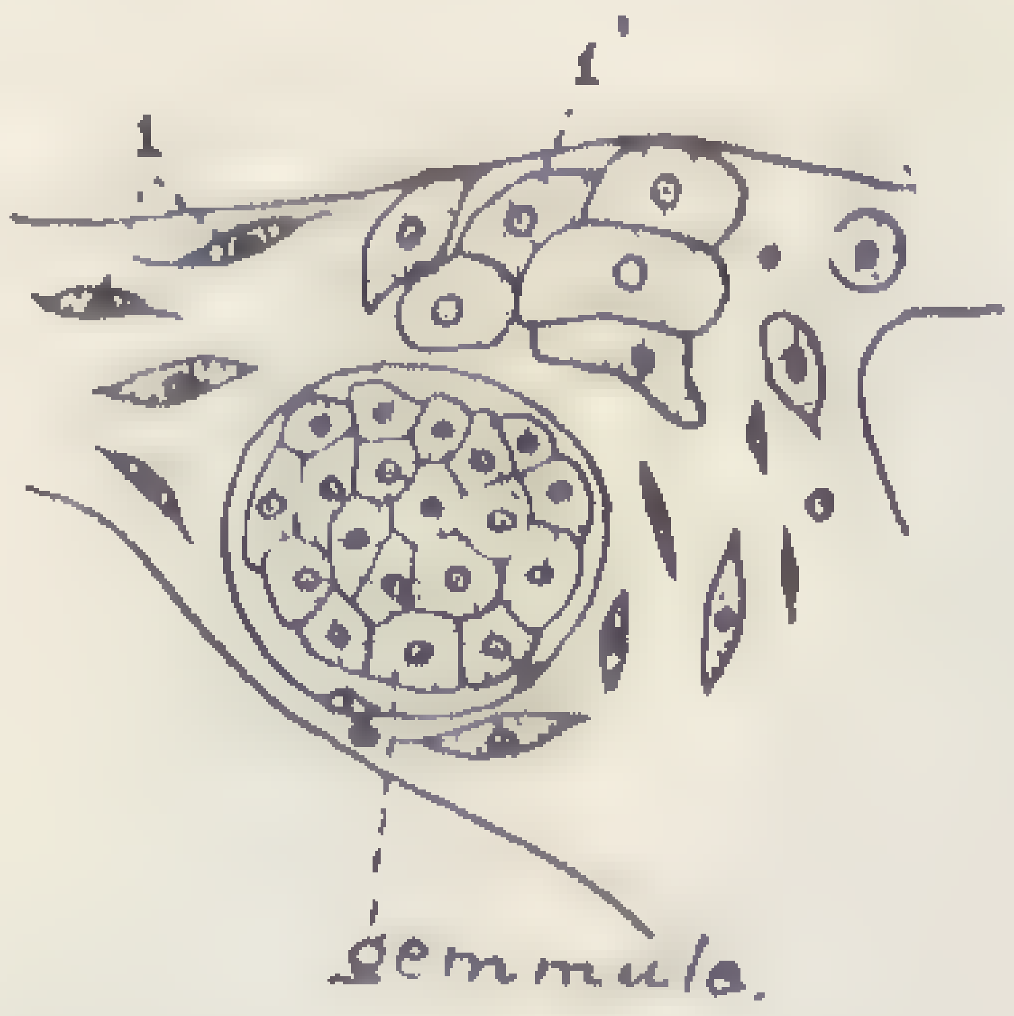

Fig. 5.

b. Next we must consider the *aggregation of mesenchyme into a superficially extended body,* — the formation of a layer. This process does not seem to be very common; one example is seen in Figure 4.

c. As the last of these processes of aggregation we have the case of *aggregation into a mass.* This wide-spread ontogenetic process may be illustrated by the formation of gemmules in a marine sponge (Figure 5). Other examples are found in the formation of the adductor muscles of

---

Fig. 3. Cross section of a rat embryo in the upper thoracic region, showing the development of the sympathetic nerve (between spinal nerve and carotid artery). From A. M. Paterson, '91, Trans. Roy. Soc. Lond., Pl. XXII. Fig. 4.

Fig. 4. Later stage of the embryo shown in Figure 1. The migrating protoplasm has aggregated itself into a layer at the surface of the embryo. See K. & H., Fig. 449.

Fig. 5. Section of a marine sponge (Esperella), showing a gemmule, a mass of aggregated mesenchyme which is about to produce a gemmule (1′), and migrating, not yet aggregated mesenchyme (1). After H. V. Wilson ('94, Jour. of Morph., IX. Pl. XIV.)

Lamellibranchs, the muscles of the foot of Gastropods (K. & H., Fig. 556, *s. m.*), and the lymph glands and spleen of Vertebrates (M., p. 414).

The reverse process to the aggregation of mesenchymatous cells is their *Dispersal*, and this has probably been brought about by the opposite cause to that producing aggregation. Since, however, this is a process taking place in a protoplasmic *mass*, its consideration must be deferred. (See page 194.)

We have been considering the different forms into which mesenchymatous elements aggregate themselves in the formation of one body; it now remains to consider the processes taking place between mesenchyme and other protoplasmic bodies. Of these processes I recognize at present four, viz.: the attachment of mesenchymatous cells to a body, following their migration thither; the encapsuling and interpenetration by a mass of mesenchyme; transportation by mesenchyme; and absorption by mesenchyme.

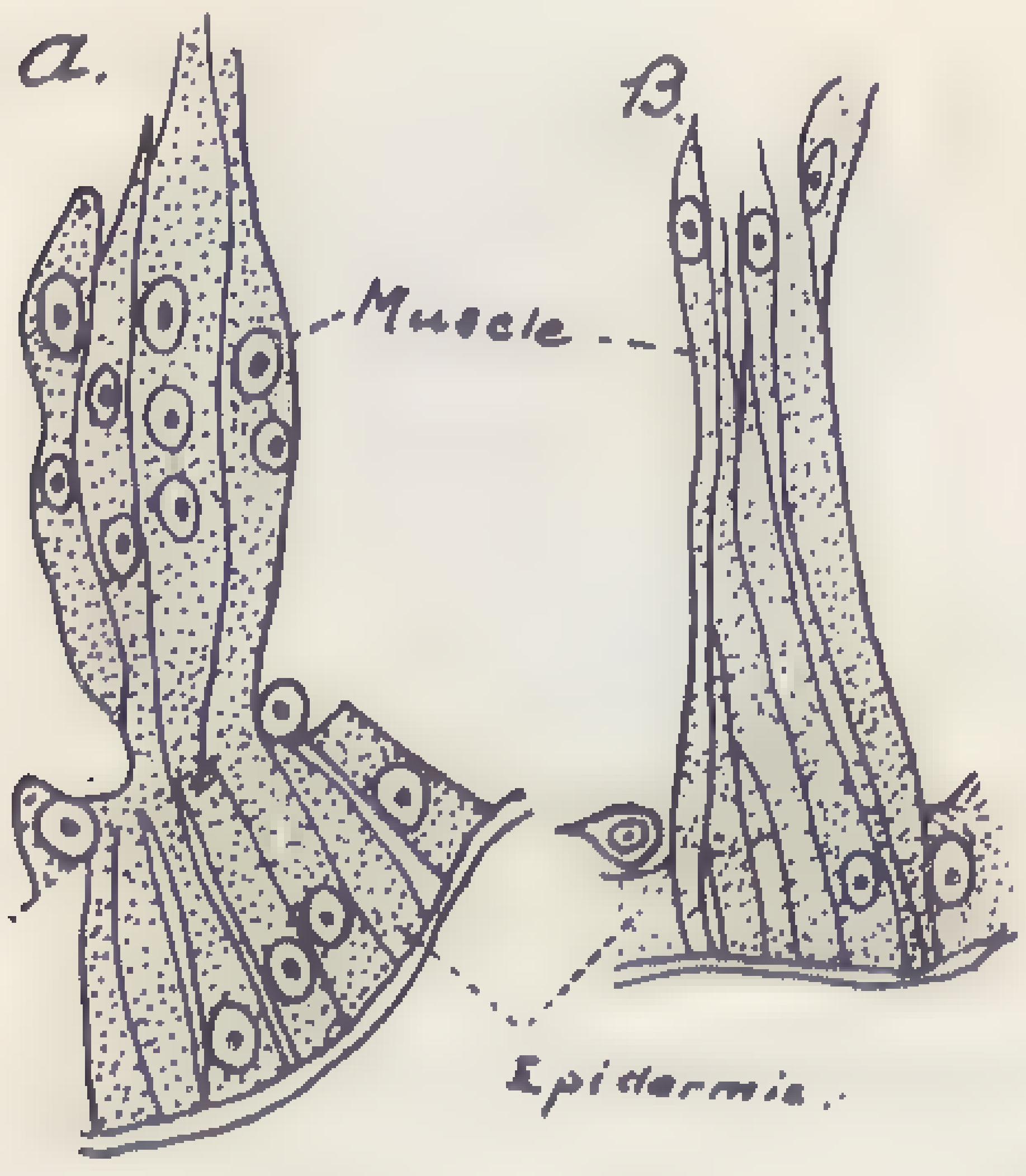

FIG. 6.

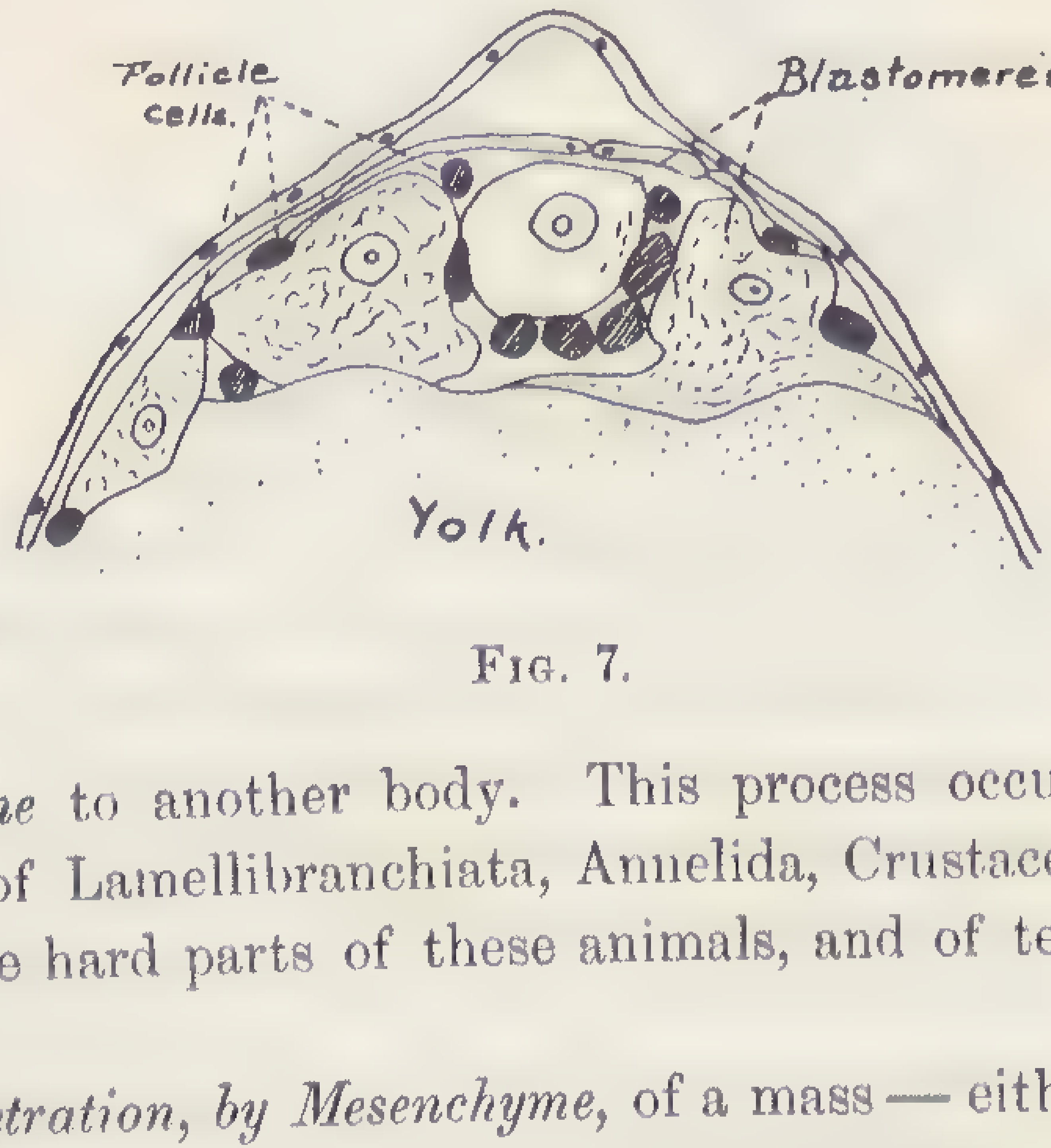

FIG. 7.

4. *Attachment of Mesenchyme* to another body. This process occurs in the union of the muscles of Lamellibranchiata, Annelida, Crustacea, and Bryozoa (Figure 6) to the hard parts of these animals, and of tendon to bone, in Vertebrates.

5. *Investment and Interpenetration, by Mesenchyme,* of a mass — either some other organ of the body or a foreign substance, like a parasite — is a not uncommon process. Especially marked is this process in the Tunicata (Figure 7), where migrating follicle cells encapsule and finally

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Fig. 6. Sections through the body wall of the Bryozoan, Paludicella; (*a*) young, (*b*) adult; illustrating the process of attachment of mesenchymatous muscles to the cuticula.

Fig. 7. Section of the germ disk of Pyrosoma, showing migrating follicular cells surrounding the blastomeres. (See K. & H., Fig. 771.)

penetrate between the blastomeres, so that it is difficult to tell which part of the embryo has been derived from the egg and which part by immigration. Compare the origin of the cutis in Echinoderms (K. & H., Fig. 195), in Mollusca (K. & H., Fig. 686), and in Vertebrates (M., Fig. 306), and of the intestinal and vascular musculature of Vertebrates (H.-M., Fig. 185).

All of these processes have this in common, that mesenchyme migrates to an organ — vessel, layer, or mass — and applies itself closely to it, sometimes even penetrating into the substance of the organ.

6. *Transportation by Mesenchyme* has hitherto been observed in but few cases. The most remarkable instance of this process is found in the Doliolidæ, where the buds produced from a stolon are transported over half the length and half the circumference of the body by means of mesenchyme cells, and are finally deposited, in very regular order, on the appendage of the budding individual. (Figures 8 and 9.) By similar means, apparently, one end of the funiculus of the Bryozoan Cristatella is transported from the dorsal to the ventral surface of the corm, as I have attempted to show elsewhere. (Bull. Mus. Comp. Zoöl., XX. 142.)

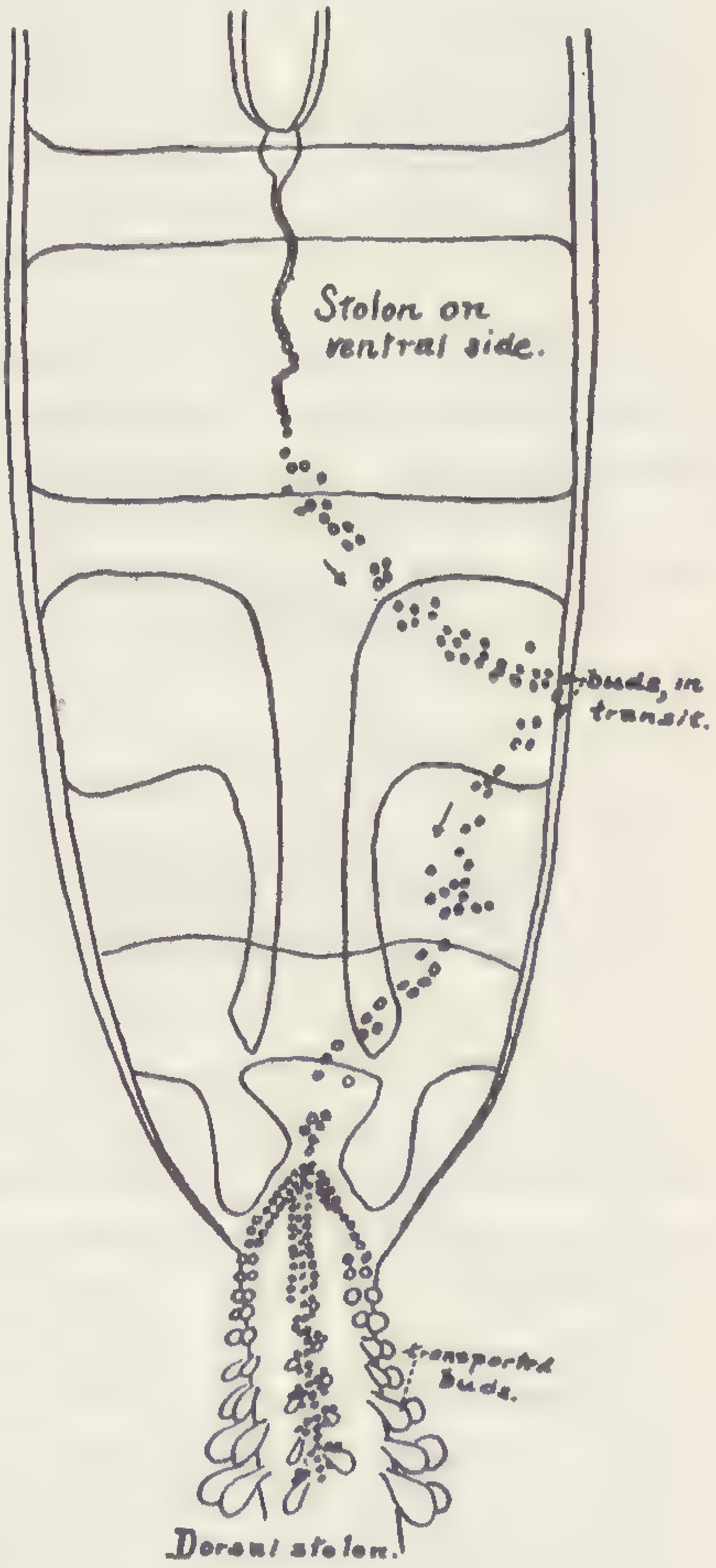

FIG. 8.

Fig. 8. Dorsal view of the posterior part of a large Doliolum "nurse." Shows the buds being transported from the ventral to the dorsal (and posterior) stolon. (See K. & H., Fig. 830.)

7. *Absorption by Mesenchyme.* Only of late years have we come fully to appreciate the great rôle played in ontogeny by the devouring capacity of mesenchyme. It is now fully established that such migratory protoplasmic bodies — phagocytes — are the most important agent in the degenerative processes which larvæ undergo in their metamorphoses.

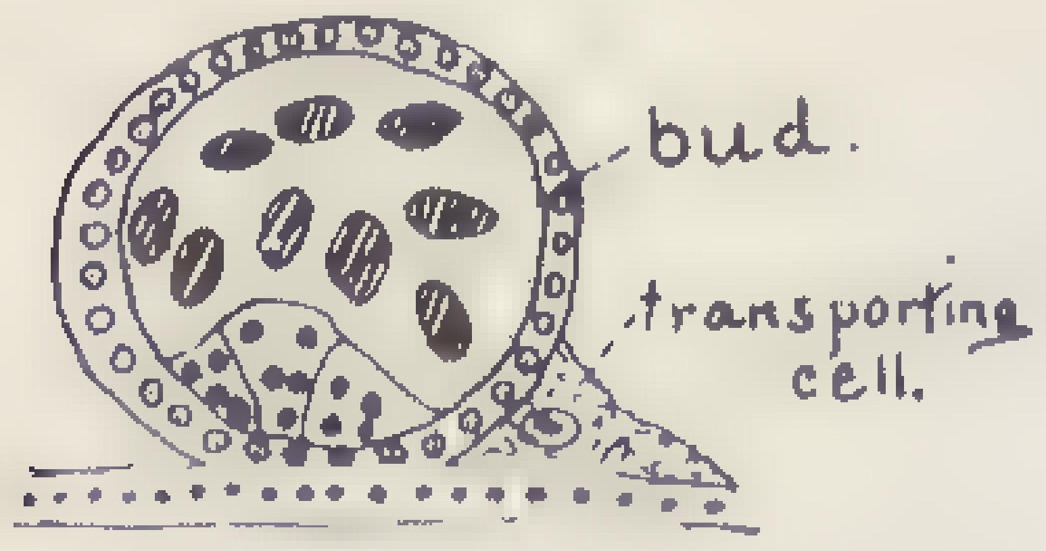

Fig. 9.

This is well shown in Insects (Figure 10), in Bryozoa probably, in Ascidians, and in the frog.

The secreting activity of mesenchyme has already been classed under general processes. No doubt mesenchymatous cells perform various other functions in the body besides transportation, digestion, and secretion, but these either have little effect on the form or concern only histogenesis.

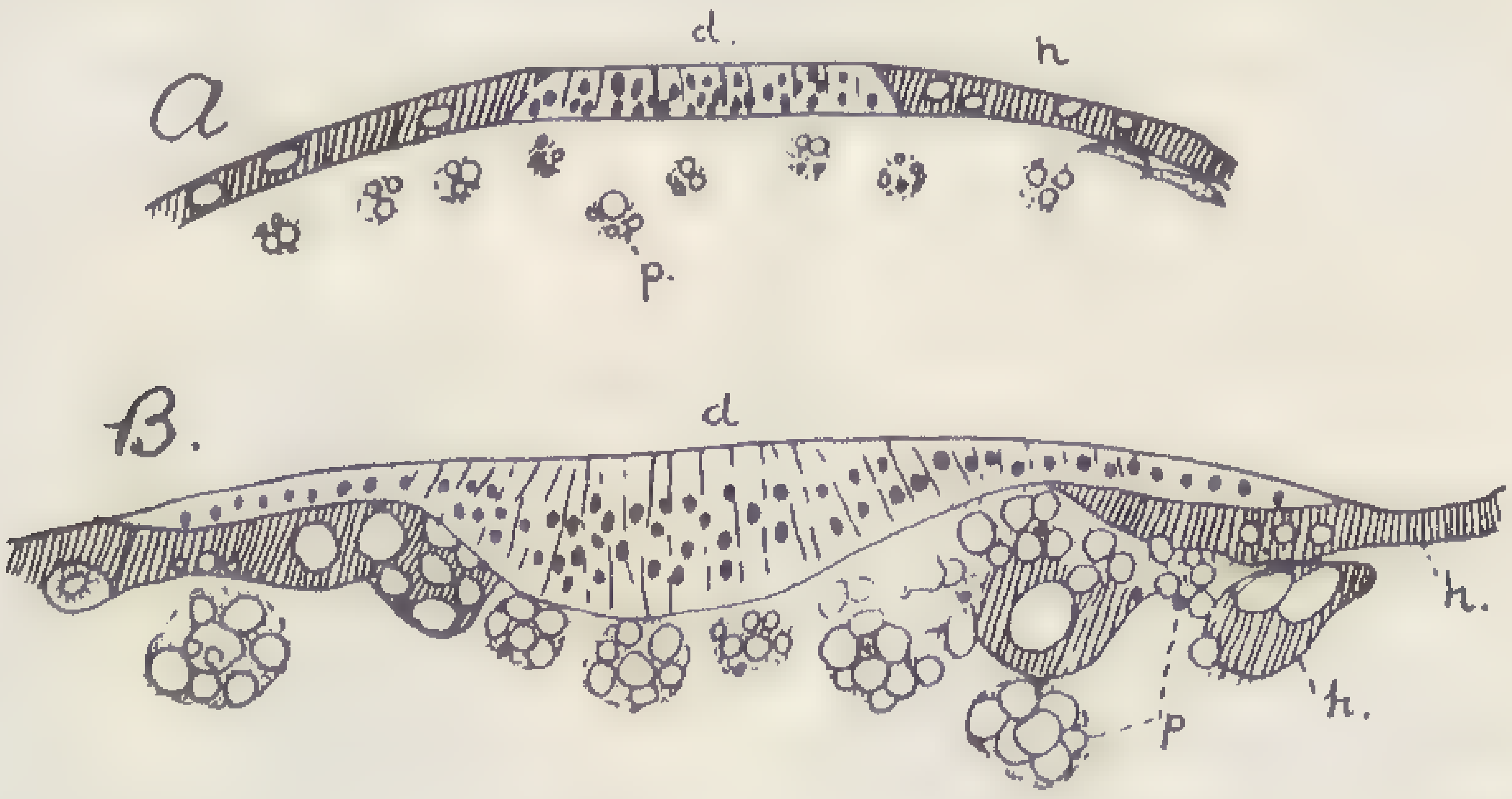

Fig. 10.

II. Ontogenetic Processes occurring in Elongated Protoplasmic Bodies — Fibres, Threads, Cords, Tubules.

Falling under this head we may recognize, first, certain general changes due to growth, such as increase in length or in thickness. These may affect either the whole body or its parts, and may lead to a diminution or increase in size.

Fig. 9. Section through the transported bud of Dolchinia, showing the amœboid transporting cell. (See K. & H., Fig. 839.)

Fig. 10. Sections through the abdominal imaginal disks of the hypodermis of Musca. *A*, from the larva. *B*, from the young pupa. In *B* the phagocytes (*p.*) are in the act of devouring the larval hypodermis (*h.*), which is replaced by the superficial growth of the imaginal disk (*d.*). K. & H., Fig. 530.

We may recognize, in the second place, certain processes which concern the *direction* which the elongated body takes and its *relation* to other bodies of the same or of a different kind. This latter group is the only one which requires further analysis and illustration. Vacuolization of cords, such as occurs in blood-vessels, will be considered in Section IV.

In this section we may consider four processes: (1) tropic processes, including the turning of elongated bodies towards or from any object; (2) the splitting of such bodies; (3) their anastomosis; and finally (4) their fusion with other organs.

1. *Turning of Elongated Protoplasmic Bodies towards or from an Object.* This is a process which has long been recognized by botanists as occurring in roots and stems, and as being a response to a stimulus coming from outside the organism. Also among hydroids the position of stolons and hydranths is often clearly determined by external stimuli. Of organs inside of the body, the determination of the direction of growth of nerves has been referred by Herbst to the action of a stimulus supplied by the organ towards which the nerve grows. Other examples of this process are not rare. I will cite a few. Herbst has already referred to the case in Turbellaria where unicellular glands of mesenchymatous origin send out long processes (the necks), which gain their appropriate connections with the other organs. So, too, the shell-gland of Crustacea, which arises from an aggregation of mesenchyme (K. & H., p. 377) secondarily sends out a stalk which makes connection at the appropriate place. The principal blood-vessels of Vertebrates arise in the area vasculosa and subsequently grow into the embryo, following certain prescribed paths (M., pp. 215, 216). From these vessels others in turn bud out, progressing towards their destined organs. According to Field ('91, Bull. Mus. Comp. Zoöl., XXI. 222), the pronephric duct of Amphibia, arising in mesoderm, grows at its posterior end, secondarily fusing with the cloaca. Again, the tubules of the metanephros, according to some authors, bud out of the blind end of the ureter, and in their further growth hit exactly the independently formed Malpighian capsules lying in the mesenchyme. In all these cases we have the elongating body clearly turning towards the object with which it is destined to unite.

Even under some abnormal conditions we have this process taking place; for instance, when a parasite lodges in any organ of a Vertebrate, new vessels are formed, which grow out towards the source of irritation. (Cf. Roux, '81, Der Kampf der Theile, pp. 150, 151).

2. *Splitting of an Elongated Protoplasmic Body* (cord or tubule). We may recognize here two sub-processes: (*a*) splitting at the tip, and (*b*) splitting along the whole length.

a. As is well known, nerves grow out as blunt stalks which repeatedly divide at their ends. Paterson has shown that some of the spinal nerves split at the somato-splanchnic angle, as shown in Figure 11. His ('88, Arch. f. Anat., Jahrg. 1887, p. 376) shows in many cases that the splitting takes place where the end of the growing nerve strikes a rigid organ. Thus, he says, when the third branch of the trigeminus strikes Meckel's cartilage, it divides into the ramus lingualis and the ramus mandibularis; and when the hypoglossal meets the jugular vein, it divides into its descending and lingual branches. The way in which the rigid organs act to produce splitting is thus explained by His. Pre-existing resistant objects may be considered as the "Motivs" of division; for as a nerve

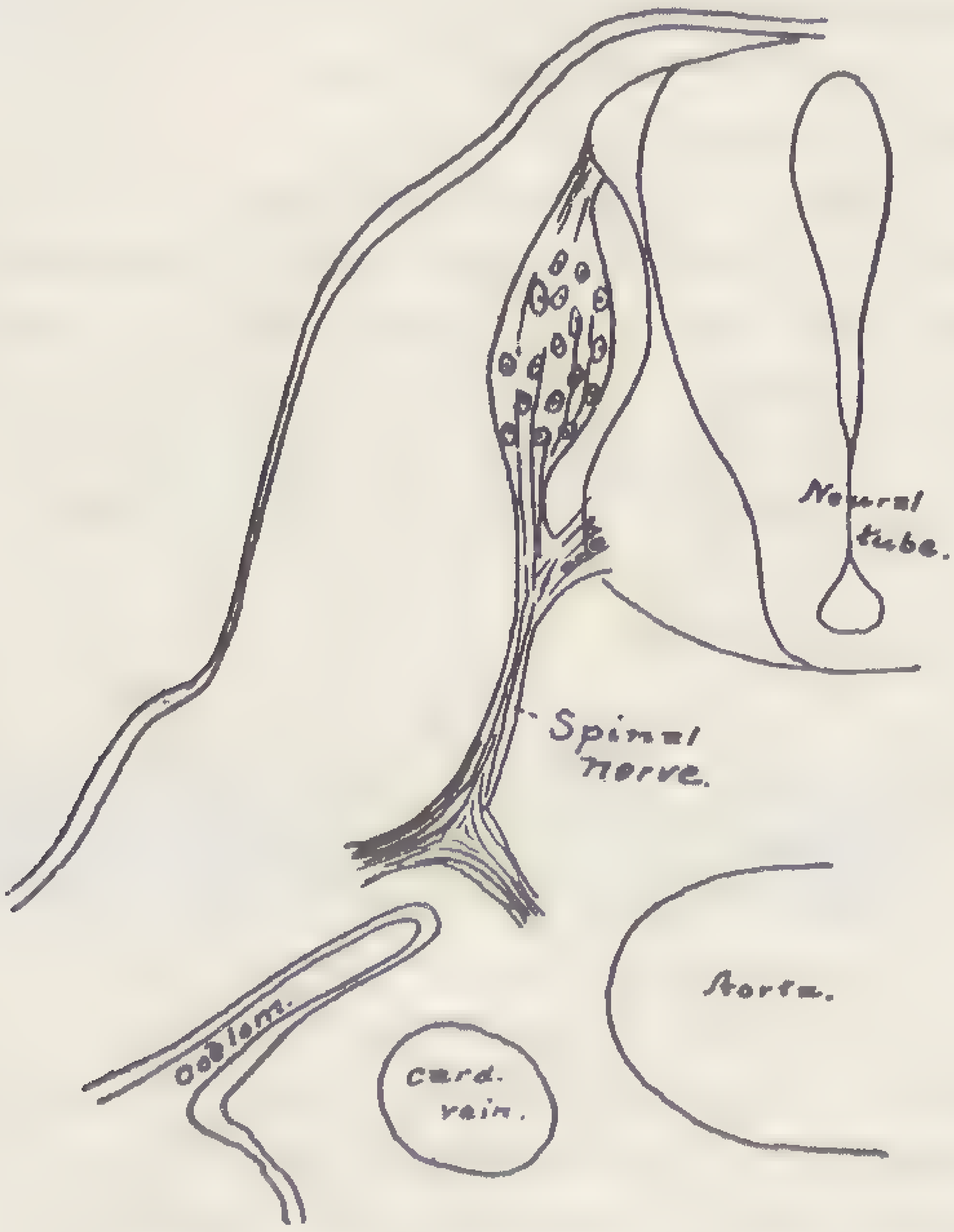

FIG. 11.

Fig. 11. Cross section of a mouse embryo in the lumbar region, showing the splitting of the spinal nerve at the upper angle of the cœlom. After Paterson, '91, Trans. Roy. Soc. London, CLXXXI., Fig. 5.

stem strikes a cartilage or a vessel, its fibres are bent in different directions and the stem divides. The resisting objects are thus considered by His to act in a mechanical, i. e. *direct* way. The results are, however, equally explicable by the response-to-stimulus theory.

The splitting which occurs at the blind ends of developing blood-vessels and excretory tubules, and the repeated divisions of many glands — salivary gland (M. Fig. 334), liver, and lungs (M., Fig. 445) — are examples of this process. Other cases are found among Invertebrates, as, for example, the tentacles of many Cnidaria (K. & H., Figs. 27, 31), "roots" of Sacculina, and liver branches of Limulus (K. & H., Fig. 338).

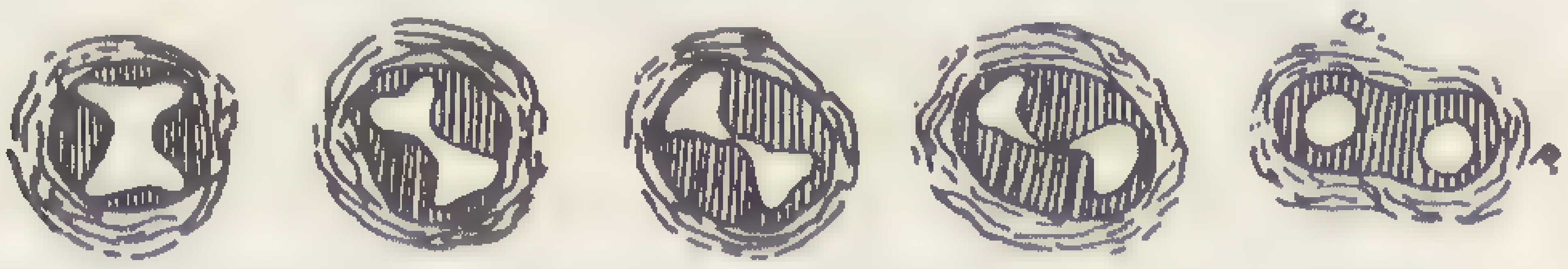

FIG. 12.

b. The second case, that of division of a tubule throughout its entire length, is illustrated in the development of the segmental duct in some Vertebrates, and in the separation of aorta and pulmonary artery. (Figure 12).

3. *Anastomosing*, or the process of fusion of similar threads, thus forming a network, is of wide-spread occurrence. It is exemplified in the development of nerves (Figure 13) and blood-vessels in Vertebrates, and in some glands, especially the vertebrate liver.

4. *Fusion with other Organs.* The process of fusion of diverse organs will be studied in greater detail in other sections of this paper. It occurs, for instance, at the close of the process of growth of the neck of a mesenchymatous gland (or other independently arising tubule) towards its insertion.

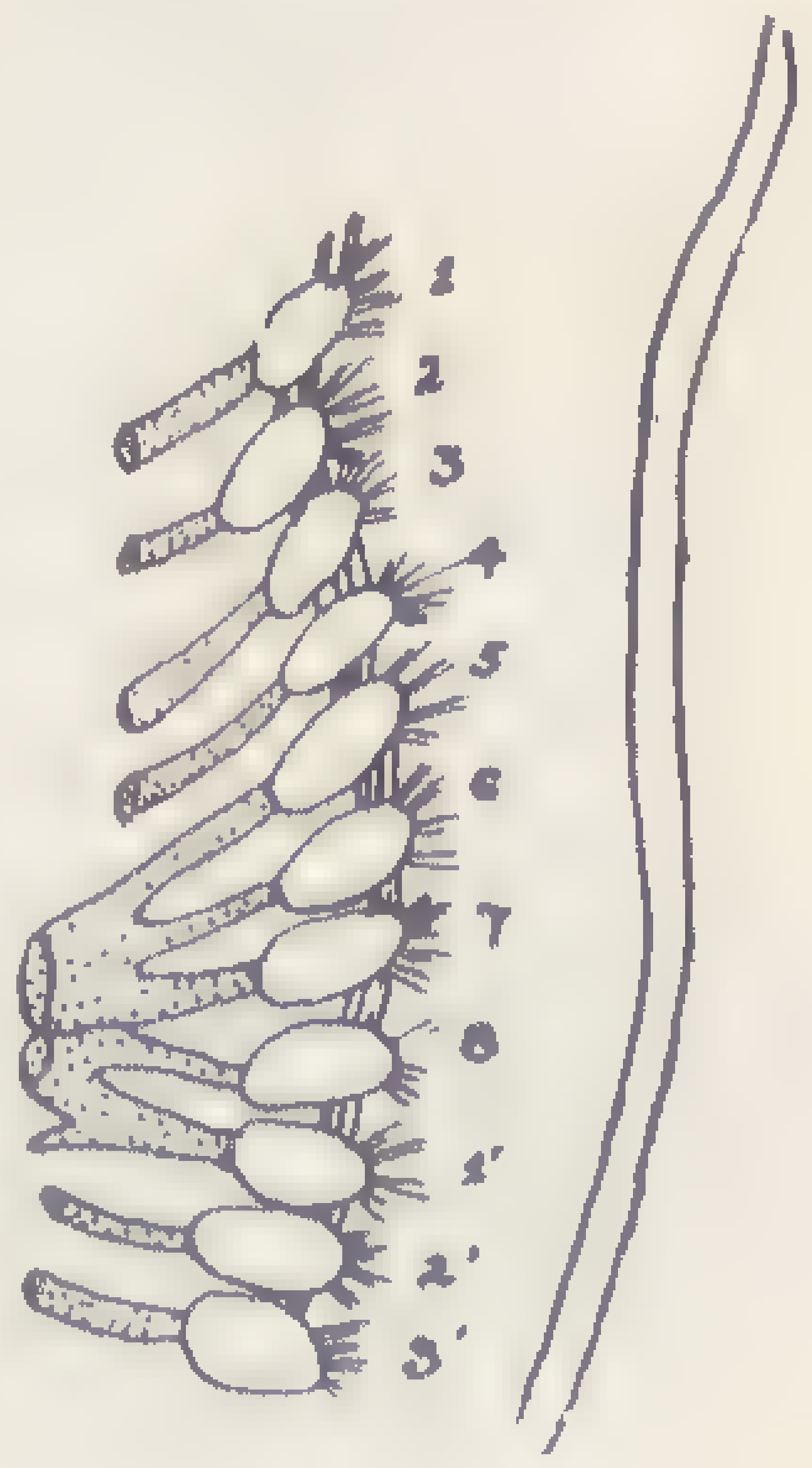

FIG. 13.

Fig. 12. Sections at different levels through the cardial aorta of a human embryo of 11.5 mm. The lowest section is at the left; the highest at the right: the separation progresses from above downwards. *a*, aorta; *p*, pulmonary artery. See M., Fig. 293.

Fig. 13. The cervical and three of the thoracic spinal nerves of a human embryo, showing the origin of the thoracic plexus. See M., Fig. 360.

III. Ontogenetic Processes occurring in Protoplasmic Layers.

The ontogenetic processes occurring in protoplasmic layers may concern one layer only, or two or more layers acting in relation to each other.

The processes which take place in one layer are principally growth processes affecting on the one hand the *area*, on the other the *thickness* of the layer.

III^a. Of the growth processes affecting *area*, we may distinguish (α) such as take place in the walls of a hollow sphere or cylinder from (β) such as take place on a plane or warped surface.

α. The areal growth occurring in a hollow sphere or cylinder (sac) may be equal in all the axes or elements of the wall, leading merely to a change of size of the sac; or it may be unequal in the various axes or elements, producing a change in form. It is this latter group which especially interests us, and it will therefore be further analyzed.

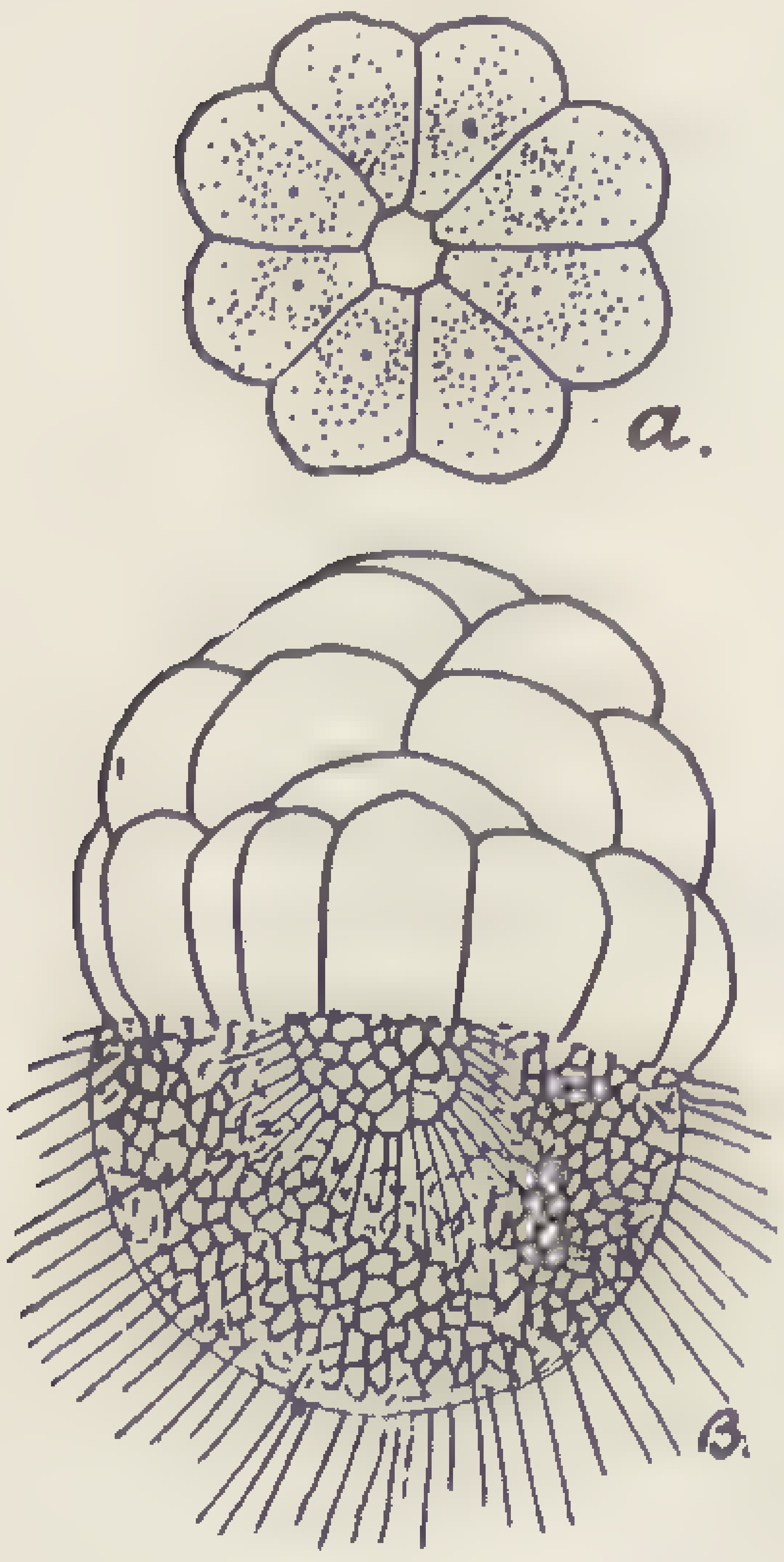

Fig. 14.

1. We may recognize three cases in the differential growth processes occurring in a sac: (*a*) unequal growth in the different axes; (*b*) unequal growth at the poles of the axis; (*c*) unequal growth in the various meridians of the sac.

These three processes deserve illustration.

a. By the process of excessive growth in a certain axis we have the ellipsoidal form produced from the spherical; as in the Sycandra larva (Figure 14); in planulæ (K. & H., Figs. 14, 30, 32, 51); in the larvæ of various worms (K. & H., Figs. 144, 158, 159); in the Echinoid blastula (K. & H., Figs. 173, 176); and in the larvæ of some Mollusca (Figs. 542, 576, 593, 596) and Bryozoa (Fig. 702).

b. Through unequal growth at the two poles of (e. g. the chief) axis, we have produced such ovoidal forms as planulæ, in which not only is the chief axis elongate, but one pole has grown more than the other. (Figure 15.) By unequal growth at the poles are produced also such

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Fig. 14. Sycandra embryos. *A*, 8-cell stage; *B*, larva. See K. & H., Figs. 2 and 3.

club-shaped pouches as the "club-shaped gland" of the Amphioxus larva (K. & H., Fig. 875).

c. Through unequal growth of the different meridians of a spheroidal or cylindrical wall we have produced from the spheroid an apparent flattening on one face, such as occurs, for example, in various cases of "epibolic gastrulation." In Figure 16 this process is illustrated in the spheroidal egg of a Heteropod. (Cf. K. & H., Figs. 66, 541, 738.)

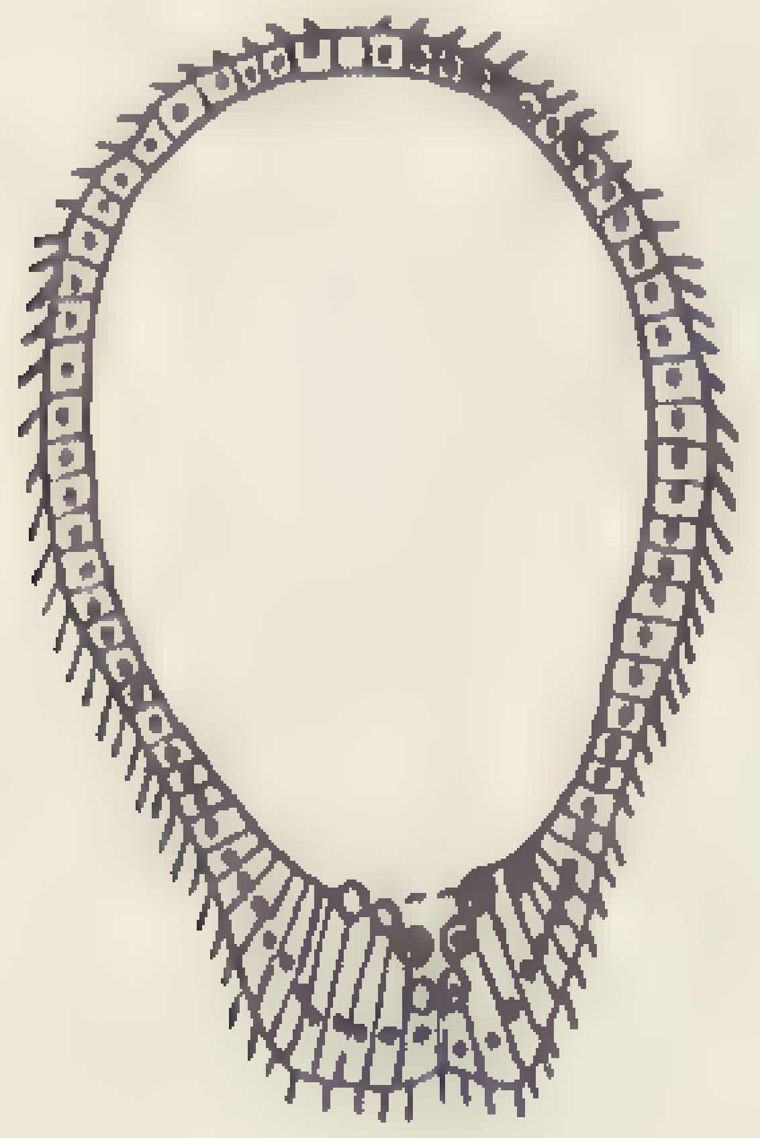

Fig. 15.

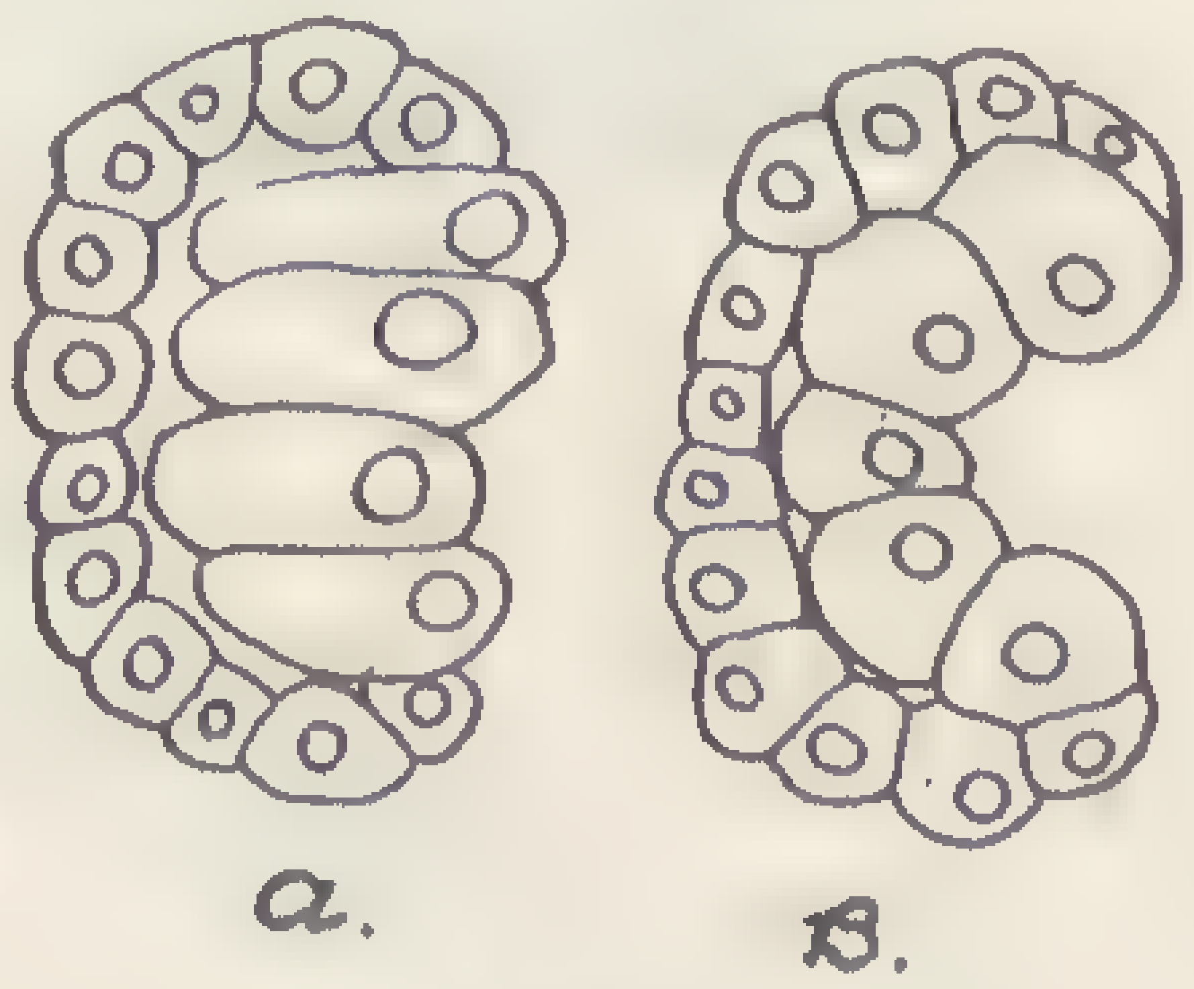

Fig. 16.

By an excess of growth along the meridians of one side of a cylinder we have produced such apparent rotations of the internal organs as occur in Crinoids and Endoprocta. (K. & H., Figs. 218, 732.)

β. Let us now consider the unequal areal growth of a part of a bounding layer taken so small that it may be considered as nearly a plane or a warped surface. We shall find that the processes taking place in such a part are among the commonest and most important of all occurring in ontogeny.

This unequal growth may result, either (2) in parts formerly lying in one plane moving into different planes; or (3) in parts formerly lying in one line moving out of that line.

2. It has long been recognized that, when in a layer excessive growth takes place over a restricted area, the result is that the excessively enlarged area can no longer occupy its former territory. Since its periphery remains relatively constant while the included layer increases

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Fig. 15. Section through the planula of Æquorea, to show the greater expanse at one pole than at the other. See K. & H , Fig. 14.

Fig. 16. Sections through young embryos of Firoloides, showing how the condition in stage *B* has been derived from that of stage *A* by greater growth along the meridians on the left than on the right. See K. & H., Fig. 584.

in area, the latter is compelled to fold, producing, according to the point of reference, an elevation above or a depression below the general level.

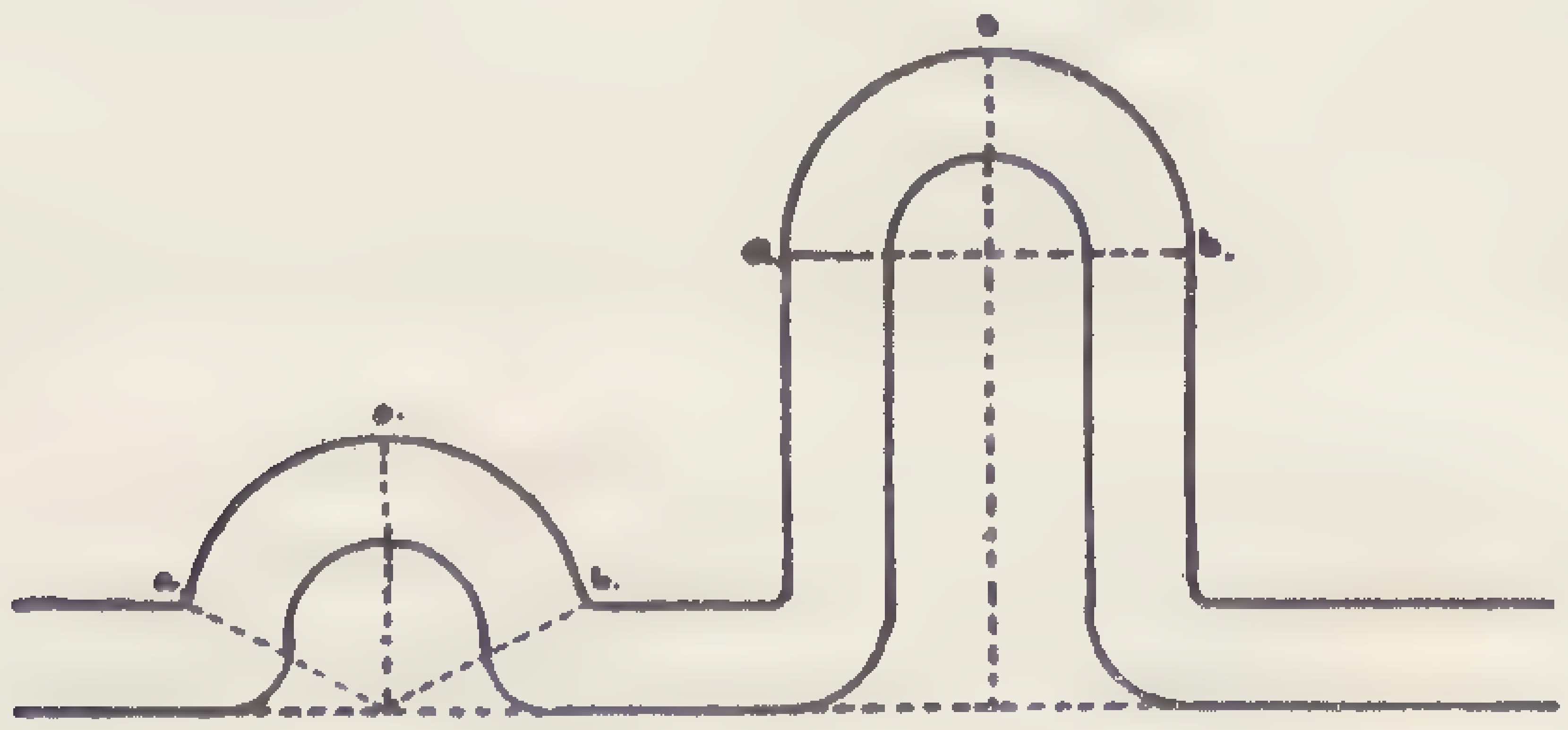

Fig. 17.

So important relatively is the process of folding that it has apparently been regarded by some authors as almost the sole developmental process. How important a rôle it plays is well illustrated in Figure 18, where (excepting the nerve masses and the heart, which are unrepresented) all of the differentiated organs of the squid which at this stage lie in the sagittal plane are represented. And these are seen to have arisen by the foldings of the unilaminar blastoderm which covers the yolk. The connections of the otocyst and the shell-gland with the exterior are now lost, but fin, mantle margins, funnel, and the various appendages of the alimentary tract, as well as the tract itself, are all seen to be arising as foldings of the blastoderm. This view does,

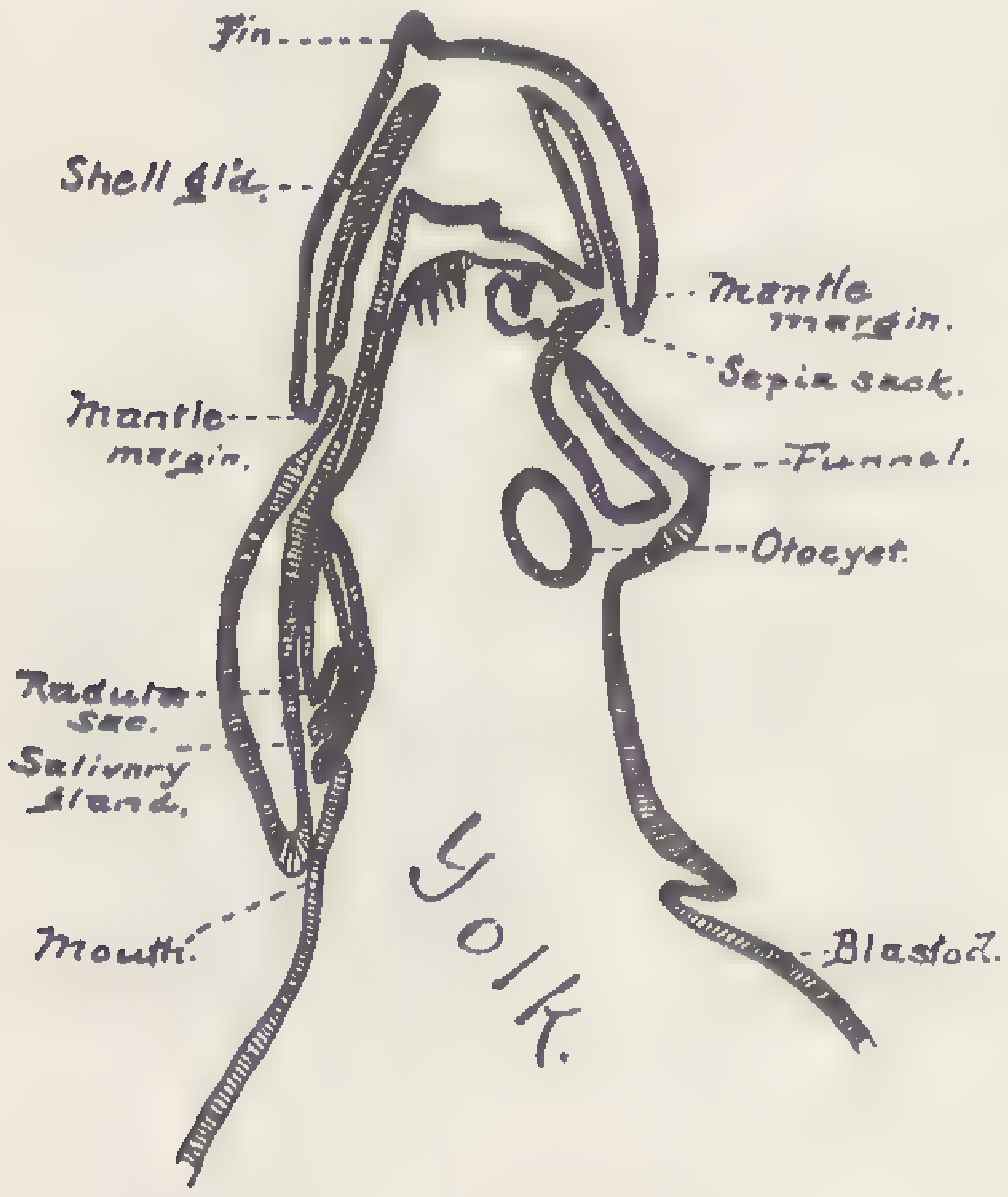

Fig. 18.

Fig. 17. Diagram representing the process of folding as it occurs in protoplasmic layers. By excessive growth between *a* and *b*, the fold *a o b* is produced.

Fig. 18. Sagittal section of an embryo of Loligo vulgaris, showing the origin of organs through foldings of the blastoderm. From K. & H., Fig. 673.

not include the lateral organs, — eye vesicles, gills, and arms, — which arise in a similar fashion as folds.

Based upon the form of the growing area, two classes of folds may be recognized: —

(*a*). *Pockets,* from a circular area.

(*b*). *Linear folds,* from an elongated area.

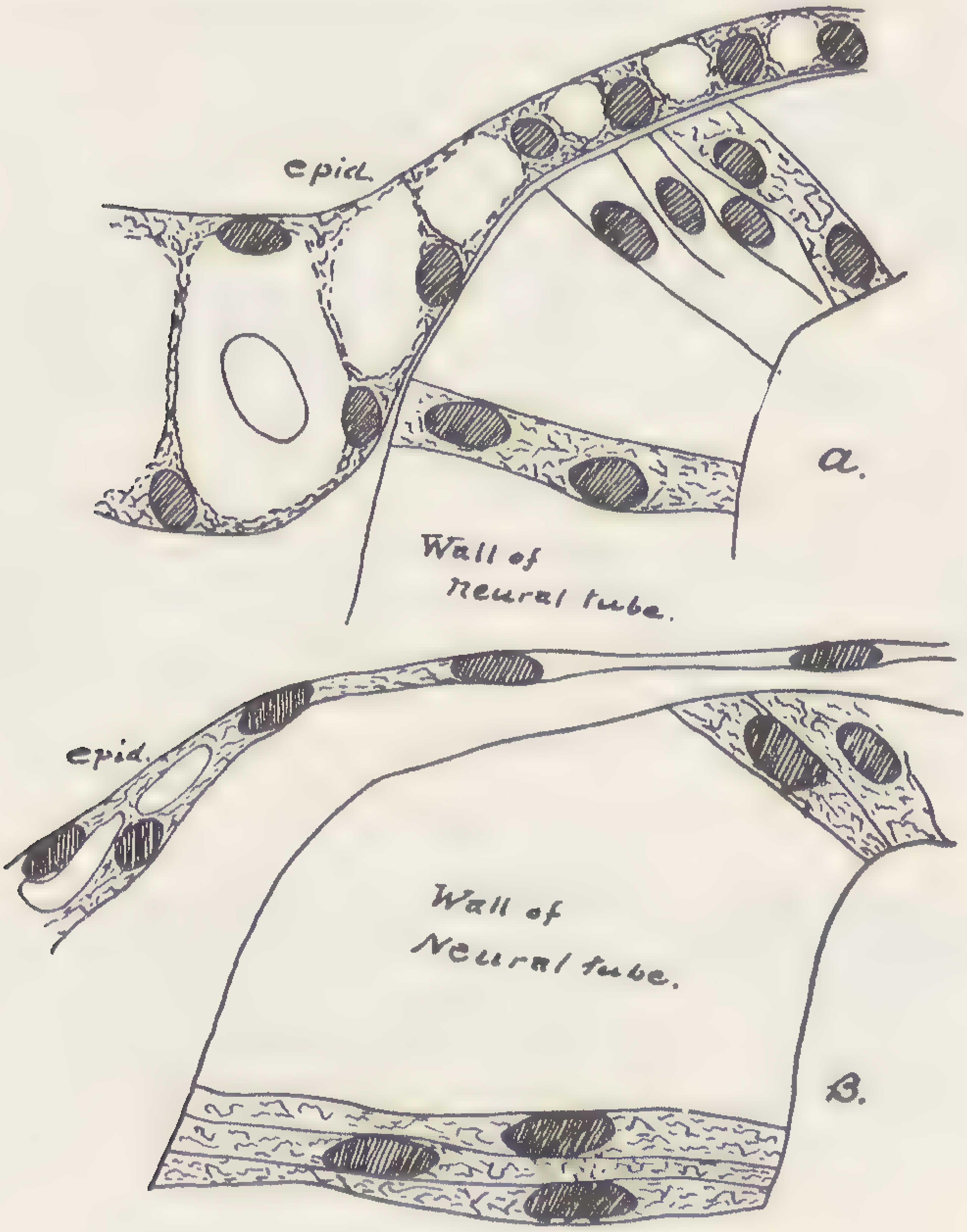

FIG. 19.

Good examples of the formation of pocket folds are seen in many cases of gastrulation, and in the otocyst, shell gland, salivary gland, and sepia sac of Figure 18.

Fig. 19. Two stages in the development of the epidermis and neural tube of the chick. Both magnified to the same extent. (From W. His, '94, Arch. f. Anatomie, Jahrg. 1894, pp. 74, 76.)

Typical linear folds occur in the formation of the neural tube of most Vertebrates, of the lateral line of fishes, and of the atrium of Amphioxus. (K. & H., Fig. 881.)

3. Inequalities of growth in different parts of the layer may take place, so that parts originally lying in a line move out of that line. By this means are produced folds lying in the plane of the layer, — folds comparable to those made by an advancing wave front on entering a bay. Such folds may occur in the *bounding line* of the growing layer, as happens, it is alleged by some, in the overgrowth of the yolk by the blastoderm, — a process by which the crescentic notch is produced on the edge of the blastoderm. Again, such folds may occur in the *midst* of a layer, producing a relative displacement of the points lying in that layer. To this process may be referred the remarkable rotation of the gill slits and mouth of Amphioxus, — the gill slits rotating from the right to the left side of the larva, the mouth from the left side towards the mid-ventral line.

III^b. The processes affecting thickness of the layer are of very common occurrence, and are of two kinds, — (4) processes leading to an increase, and (5) those leading to a diminution in thickness. These variations in thickness may be general, extending over a considerable part or the whole of the area under consideration, or they may be restricted to a small part of that area.

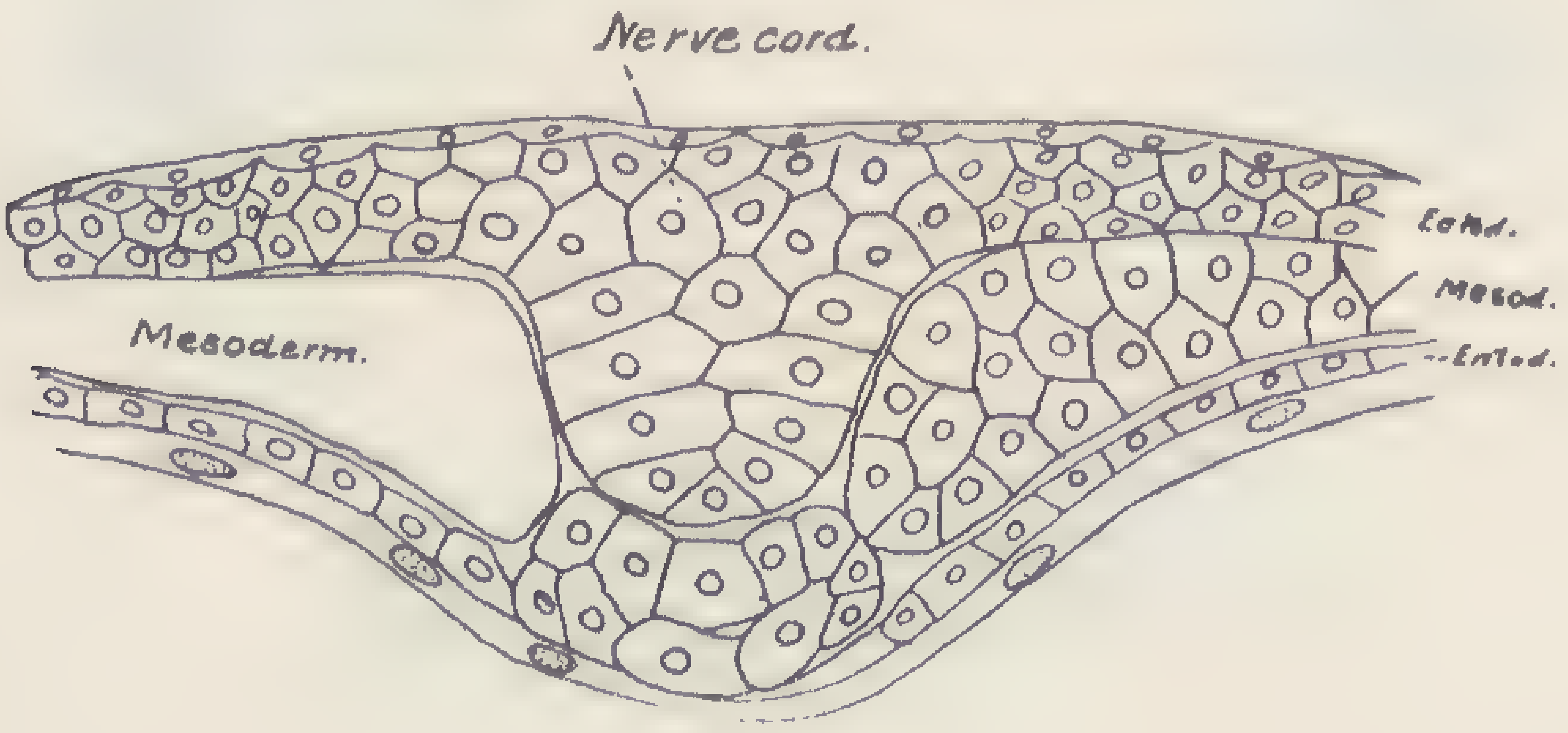

Fig. 20.

4. The process of thickening is illustrated in the lateral walls of the neural tube of Vertebrates (Figure 19), and, localized, in the formation of the neural tube in Teleosts (Figure 20). Other examples are seen in

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Fig. 20. Cross section of the blastoderm of a bony fish (Serranus atrarius) to show the linear thickening of the ectoderm to produce the nerve cord. (From H. V. Wilson, '91, Bull. U. S. Fish Commission, IX. Plate XCV.)

the papillæ which precede the formation of feathers, and in the ingrowing follicles which form hairs.

5. A general thinning of the walls of the organs is found to accompany the development of many Invertebrates. Figure 21 illustrates this fact.

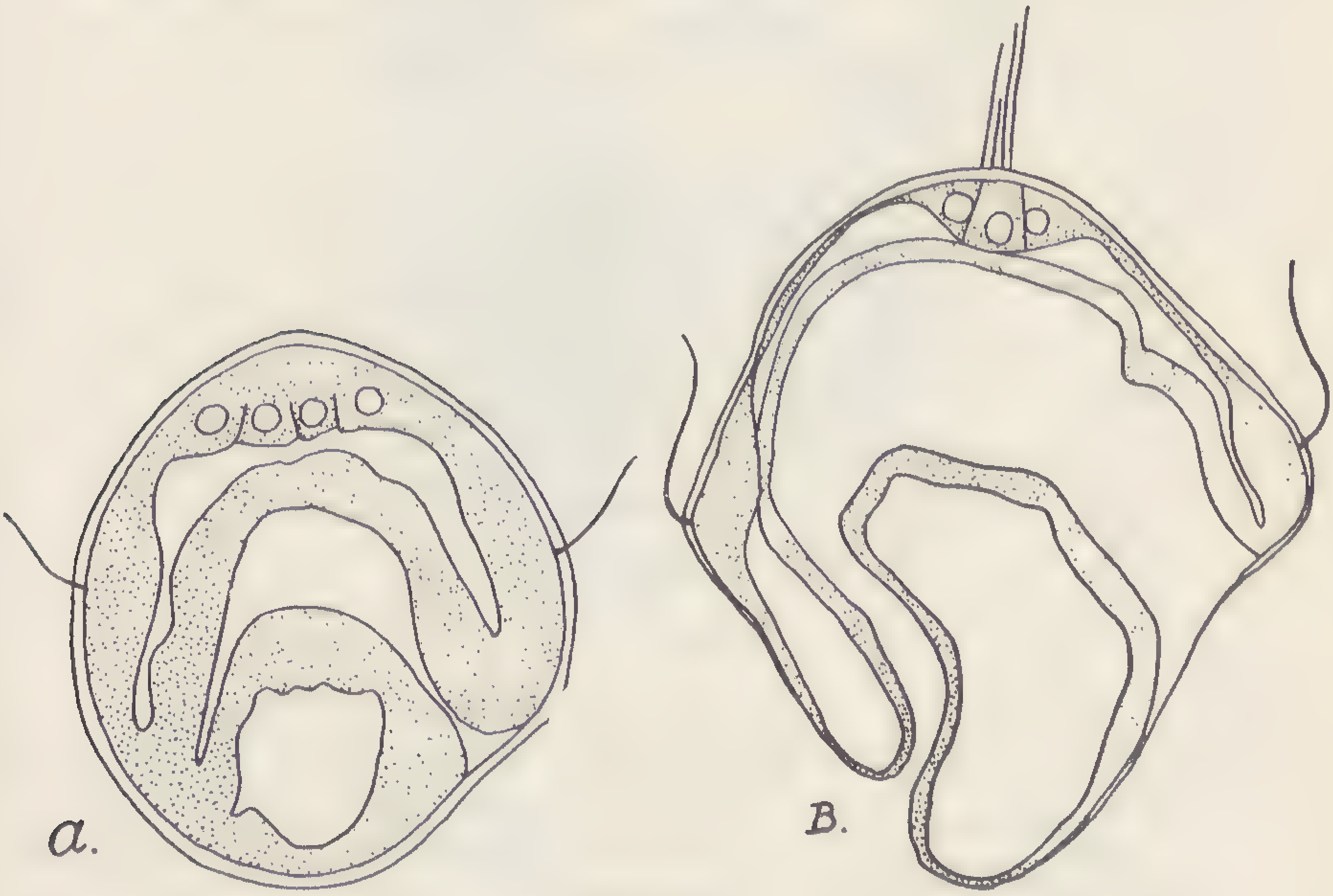

FIG. 21.

In Figure 19 a general thinning of the epidermis is seen in passing from stage *a* to stage β. A good example of local thinning is seen in the formation of the ependyma of the roof of the brain and medulla.

The processes of thickening and thinning may go on side by side, as in the formation of the lens of the vertebrate eye (Figure 22), where the outer and inner layers of the lens are at an early stage of nearly equal thickness, but where, in the later stages, the outer layer becomes thin, and the inner layer enormously thick.

III^c. Next, we must consider the processes by which a single continuous layer experiences an interruption in its continuity. This may take place (6) by a complete *atrophy* of a part of the membrane, thus forming a hole or rift in it, or (7) by a separating off of a certain piece from the membrane.

Fig. 21. *A*, younger, and *B*, older Trochopore stages of the annelid Eupomatus uncinatus, seen in sagittal section. Drawn to same scale. Outlines copied from Hatschek, '86, Arb. Zool. Inst. Wien, VI. See also K. & H., Fig. 118.

6. The former process occurs typically in the ontogeny of reptiles, and perhaps of mammals, where the middle of the layer of "primary entoderm" roofing the yolk cavity atrophies, making a great hole in the layer.

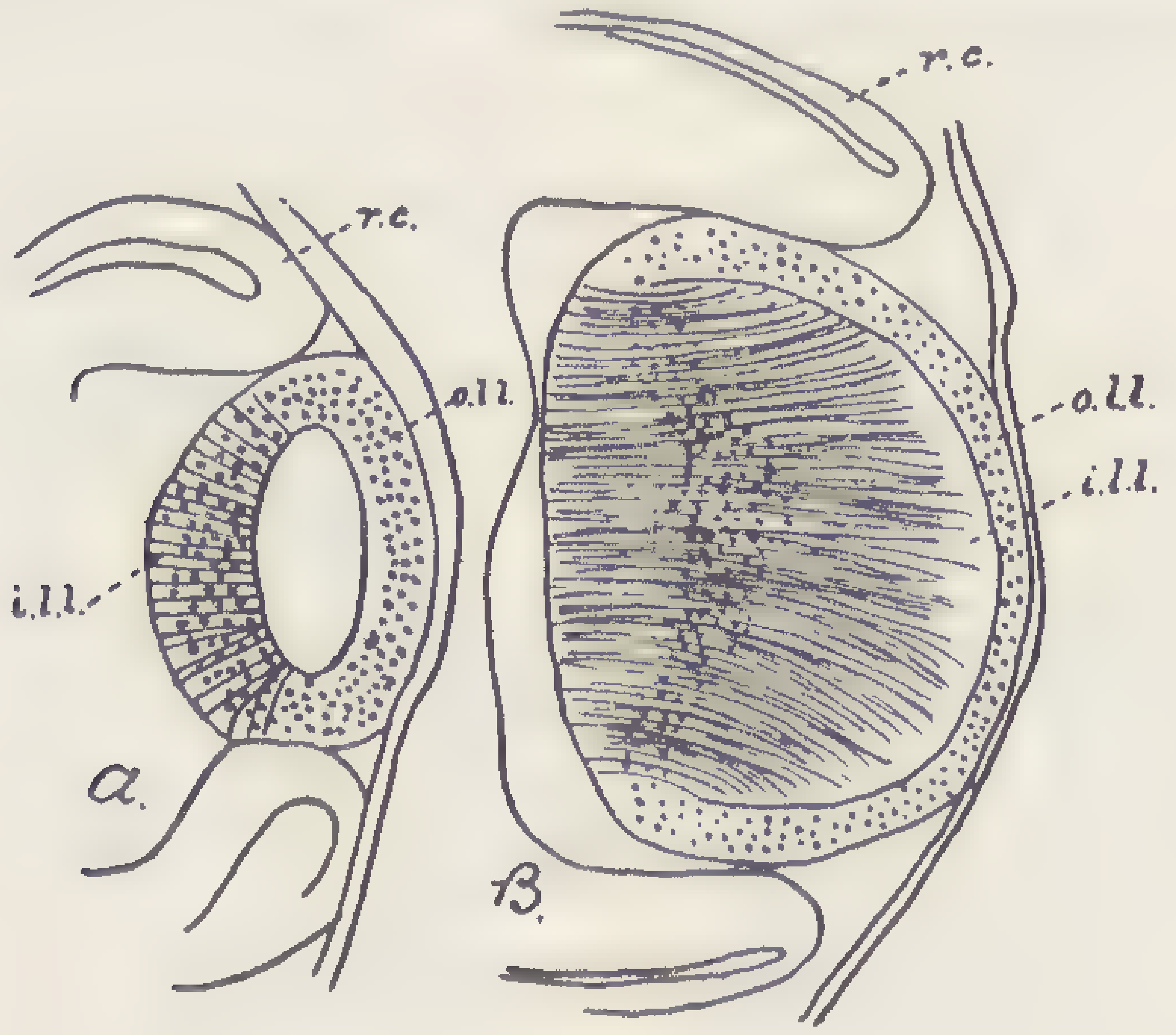

FIG. 22.

7. The latter process is represented in Hatschek's familiar figure of the formation of the neural tube of Amphioxus (Figure 23), where the medullary plate is shown as falling below the general level without the intervention of a fold. Compare the formation of the "body plate" of the Nemertine Lineus, K. & H., Fig. 105, and gastrulation in Apis, K. & H., Fig. 495.

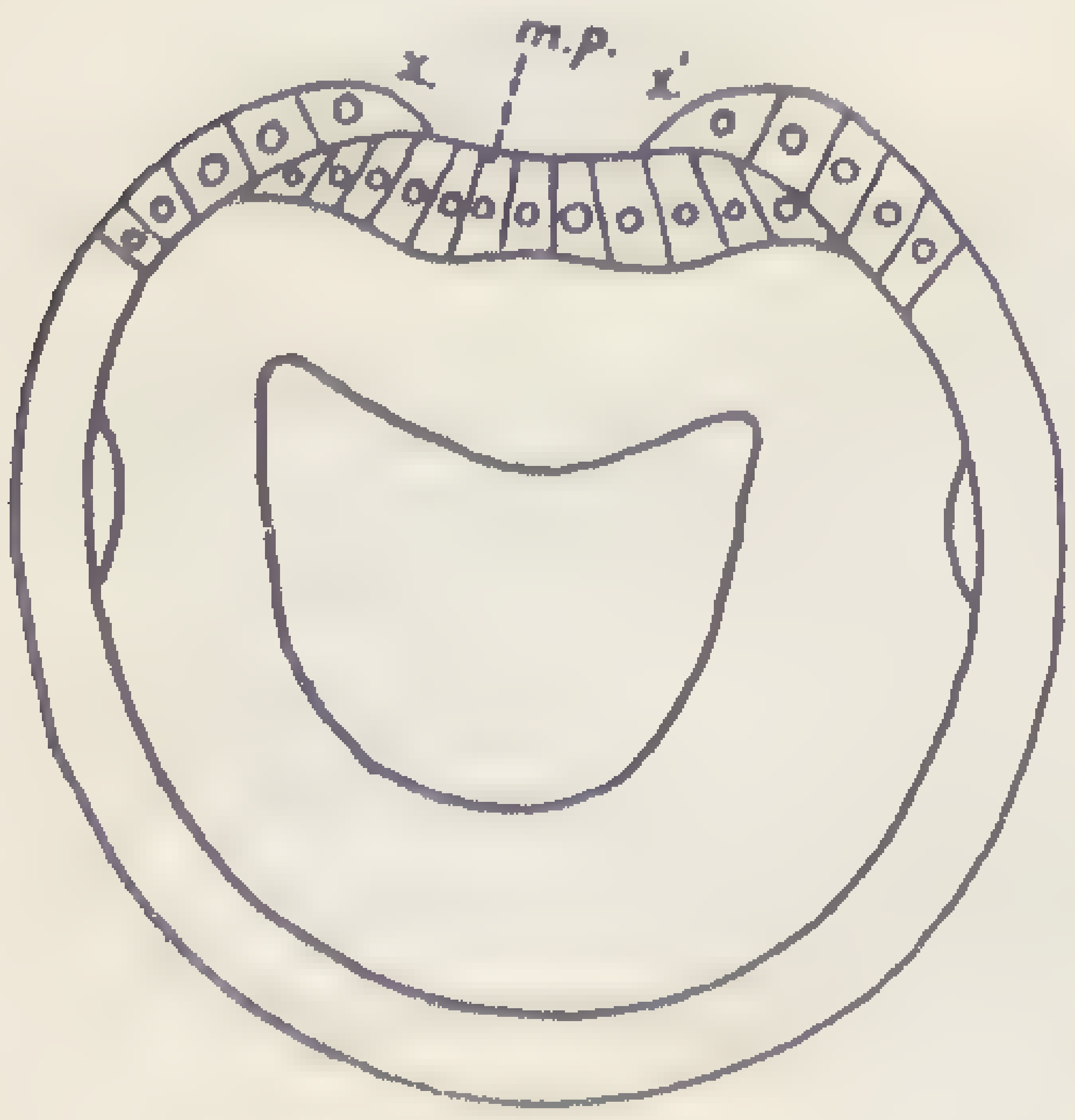

FIG. 23.

III^{d}. Finally, we must consider the important processes involving two or more protoplasmic layers. Such layers may move either towards or from each other. We will here consider only the former group of cases.

8. The mutual approach and fusion of layers may be called *con-*

Fig. 22. *A.* Section of eye of chick embryo on third day. (See M., Fig. 413.) *B.* Section of eye of rabbit embryo, 13 days old. (See M., Fig. 409.) *i. l. l.*, inner lens layer; *o. l. l.*, outer lens layer; *r. c.* retinal cup.

Fig. 23. Section across an Amphioxus embryo, showing the medullary plate, *m. p.*, which has sunk below the level of the ectoderm, producing a discontinuity in the latter. From H.-M., Fig. 69.

crescence. We may distinguish three sub-processes under this head,

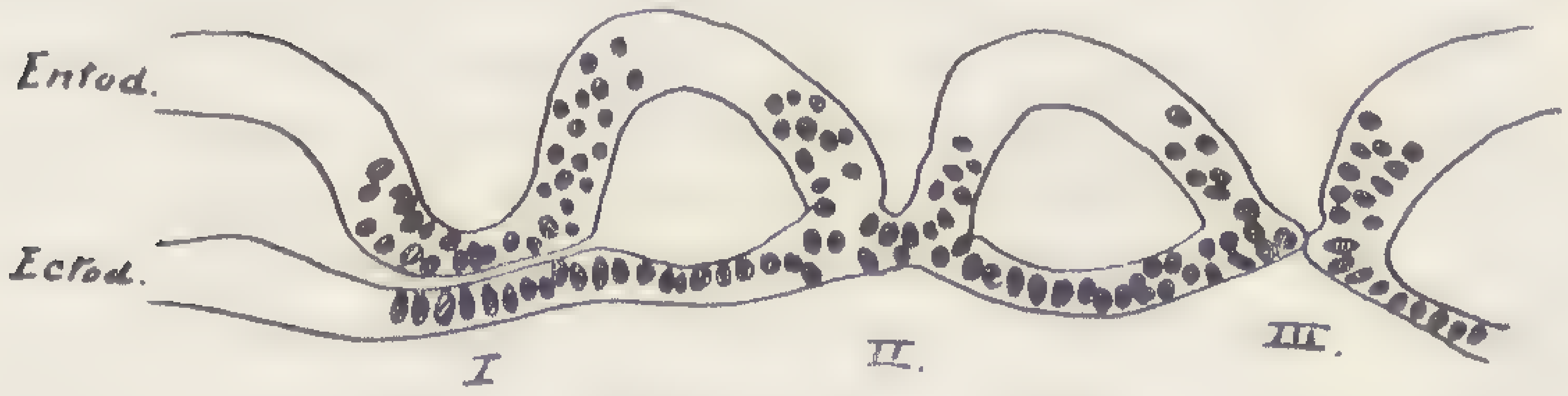

FIG 24.

depending upon whether (*a*) the concrescence takes place along the free margins of layers, or (*b*) along their surfaces, or, finally, (*c*) along the edge of folds. This concrescence is usually quickly followed by other processes which we will consider later.

a. The concrescence of layers by their free edges is illustrated in the cases of the growing together of the free edges, *x* and *x*′, of the ectoderm in the Amphioxus embryo at a stage a little later than that shown in Figure 23. (K. & H., Fig. 504.)

b. The concrescence of layers flatwise is illustrated in the formation of the vertebrate mouth when the anterior end of the entodermal sac comes in contact with the ectoderm. Likewise in the formation of the gill slits of Vertebrates the broad bottoms of the entodermal sacs move to the ectoderm. (Figure 24, I, II.)

c. Concrescence along the edges of two folds is perhaps the commonest of these three forms of concrescence. It is that by which in Vertebrates the neural tube is closed (Figure 25, *A*, *B*, *C*) ; ectodermal

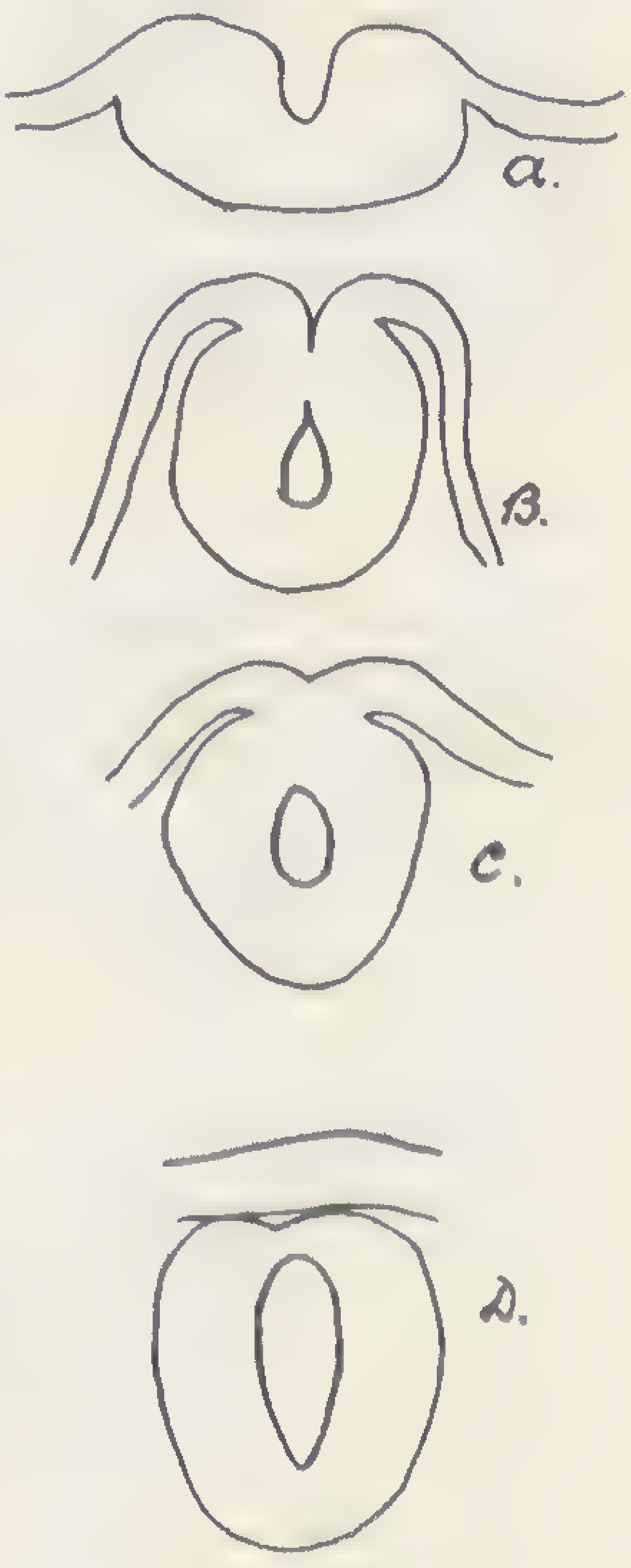

FIG. 25.

Fig. 24. Part of a frontal section through an embryo of Acanthius vulgaris, of about the stage of Balfour's Stage I. Shows 3 stages in the formation of the gill slit, I, II, illustrating *concrescence* of layers flatwise ; III, *perforation*. Original. From a preparation kindly lent me by Mr. H. V. Neal.

Fig. 25. Cross sections through the neural tube of embryo frogs of different ages, showing the concrescence of the lips of the medullary groove (*A*, *B*, *C*), and (*D*) the final separation of the upper and lower layers of the fold. After H. H. Field, '91, Bull. Mus Comp. Zoöl., XXI. No. 5.

pockets, like the lens and otocysts, become transformed into closed sacs; and grooves, like those of the lateral line, become transformed into canals. In Invertebrates also this process is a very common one, being exemplified in the closure of the blastopore, in the closure of the amniotic cavity in Insects (K. & H., Figs. 474, 475, 484), and in the formation of optic and otic vesicles generally (K. & H., Figs. 377, 630, 681–683).

The end of the process of concrescence proper is a *fusion* of the two concrescing layers, whether the concrescence is occurring along free edges, flatwise, or along the edges of folds. (Fig. 25, *C*, Fig. 24, II.)

9. Frequently this process is followed by another one; viz. the *perforation* of the fused layers (Fig. 24, III), or the *separation* of the upper and lower components of the folds when two folds have been concrescing (Fig. 25, *D*). By means of *perforation* the two spaces separated by the fused walls are put into communication with one another. By means of *separation* a pocket becomes a closed sac, and a groove becomes a tube.

IV. Ontogenetic Processes occurring in Protoplasmic Masses.

These may be classed into three categories according as the most *prominent* change produced is (IV^a) in volume, (IV^b) in form, or (IV^c) in number of masses.

IV^a. 1. Under the first group are included changes produced by *growth* which is not uniform in all parts. Thus the growth may be prevailingly along one axis, by which means a cylindrical mass is derived from a spherical one (embryos of Dyciemidæ, K. & H., Fig. 99), or it may be excessive at one pole (gemmules of sponges, H. V. Wilson, '94, Jour. of Morphol., IX., Pl. XVI.), or along one meridian. Again the growth may be more localized, being confined to a small area or to a line; as, for instance, in the case of the mesodermal core of the appendages of Arthropods (K. & H., Fig. 371), and of Vertebrates (Figure 26, "limb-bud"). By

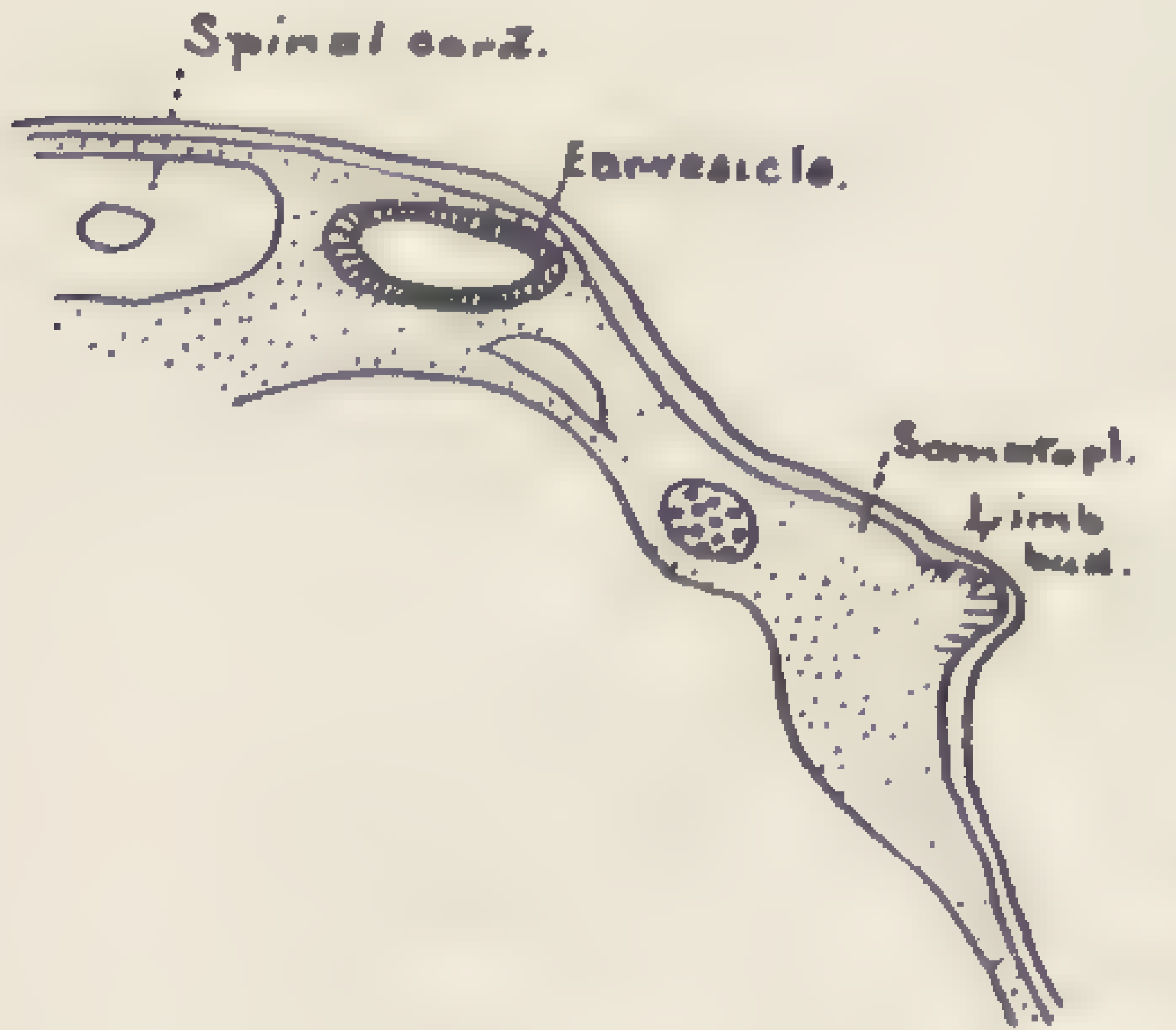

Fig. 26.

Fig. 26. Cross section through embryo of a Teleost, Fundulus, showing origin of the pectoral limb-bud as a solid outgrowth of the somatopleure. After E. R. Boyer, '92, Bull. Mus. Comp. Zoöl., XXIII. No. 2, Fig. 58.

this process of localized solid growths the principal differentiations of Phanerogams occur.

IV^{b}. 2. An important change of form of a protoplasmic mass may occur independently of growth by a *rearrangement of the nuclei* of the mass.

Good examples of this process are found in the development of the larva of Lucernaria (Figure 27); in the development of Ctenophores (K. & H., Fig. 67); and in the changes of form occurring in the "ectodermal basal plate" of Salpa (an apparent syncytium), according to the figures of Heider ('95, Abh. Senckenberg. naturf. Ges., Bd. XVIII. Figs. 32, 40, 41). In so far as this process involves the migration of nuclei, it is clearly closely related to Process I. 1.

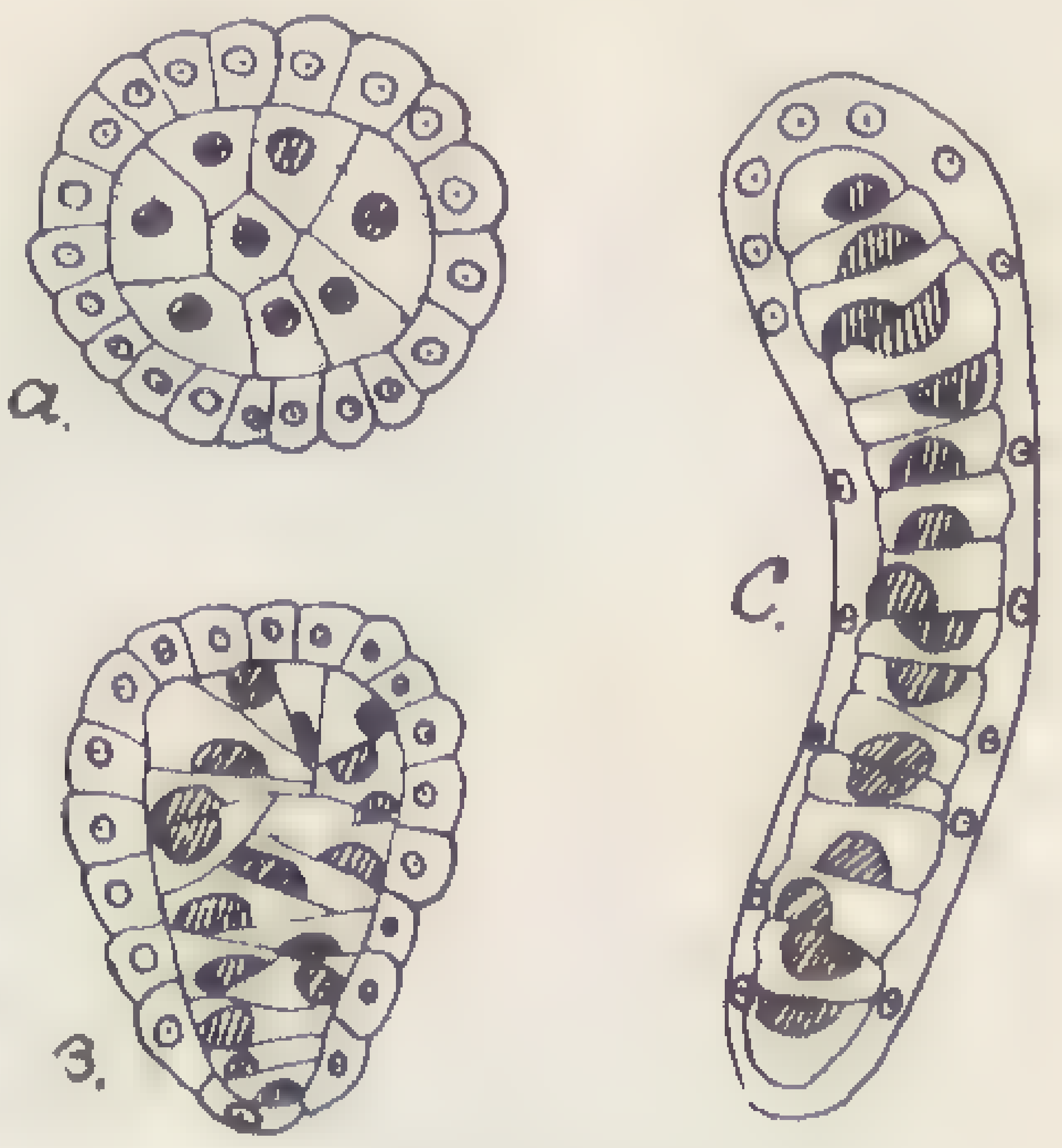

FIG. 27.

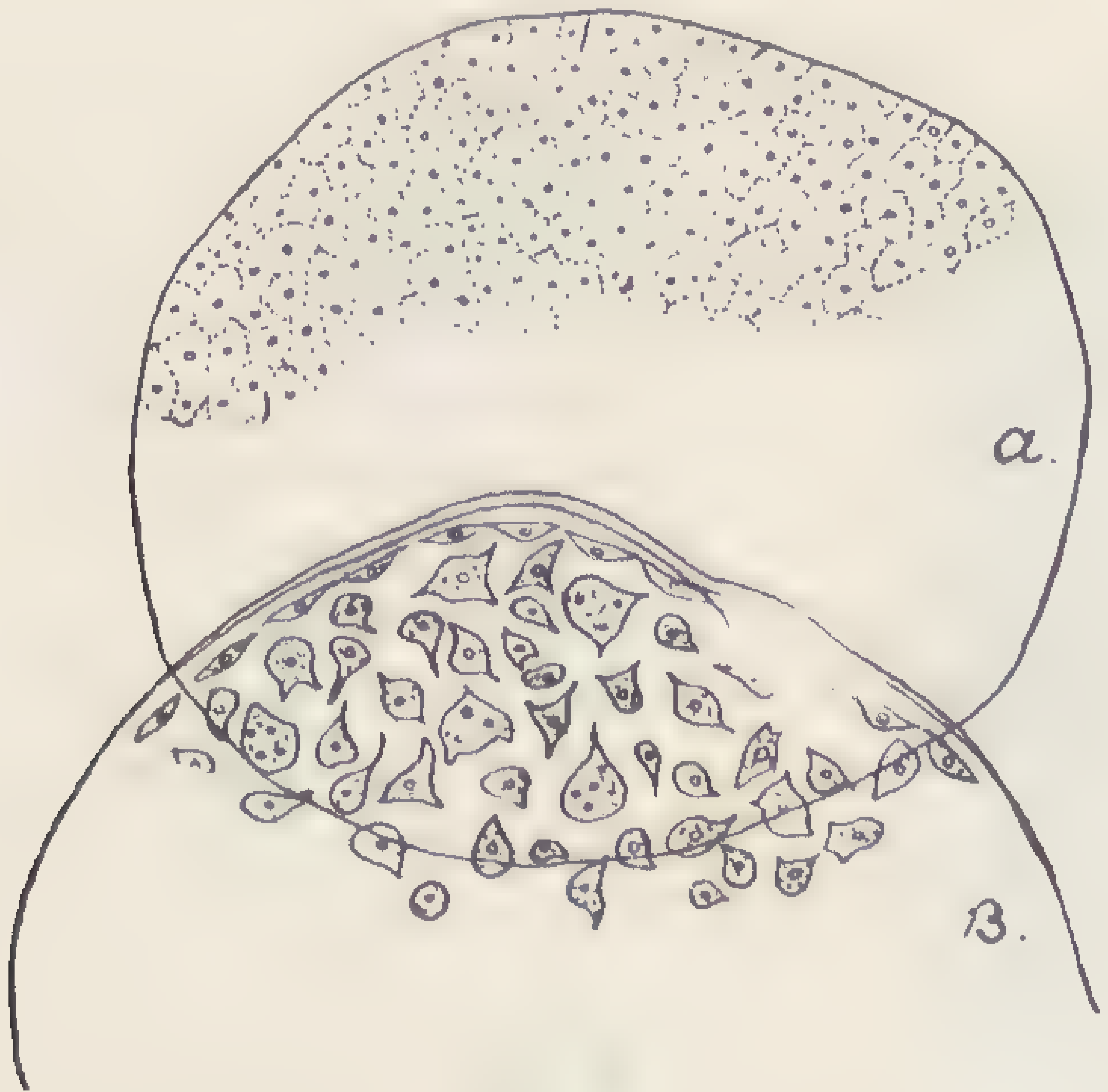

FIG. 28.

Fig. 27. *A*, *B*, *C*, are three successive ontogenetic stages of Lucernaria. The transformation from stage *B* (36 nuclei in the section) to stage *C* (33 nuclei in the section) is due to a rearrangement of the nuclei. See K. & H., Fig. 49.

Fig. 28. *B*, dispersal of elements of the gemmule *A*, which was formed, as illustrated in Figure 5, by the aggregation of similar mesenchymatous elements. This process precedes the development of the gemmule into a larva, and accompanies the imbibition of water by the gemmules. After H. V. Wilson, '94, Jour. of Morphol., IX., Plate XVI.

3. Another way in which the mass changes form without growth is by *vacuolization.* Through vacuolization there arise, e. g., the blastula in many eggs, the secondary body cavity in many Invertebrates (K. & H., Fig. 131, 689), and in Bryozoa the cavities of the bud. This process of vacuolization, which I introduce for convenience at this place, is not confined to masses. Many cords by vacuolization (canalization) become tubules, and many layers become divided into two. Of vacuolated *cords,* I need refer only to the formation of capillaries in Vertebrates and of nephridia in Invertebrates; of vacuolated *layers,* to the origin of the cœlom in most Vertebrates. In all cases, the acquisition of a mass of water at the centre causes a rearrangement of the nuclei.

4. Perhaps this is the most fitting place to mention the process of *Dispersion* of elements, which occurs not only in bodies originally formed by aggregations of mesenchymatous elements (Fig. 28), but also in *layers* having an epithelial origin, e. g. the ectoderm of Distomum. (K. & H., Fig. 88.)

IV^c. The remaining processes occurring in protoplasmic masses are of such a nature as to alter the *number* of masses. We can distinguish, correspondingly, two classes: the first including such as have to do with the formation of two masses from one, through division; the second including those which have to do with the union of two masses into one. The first, then, are *division* processes; the second, *fusion* processes.

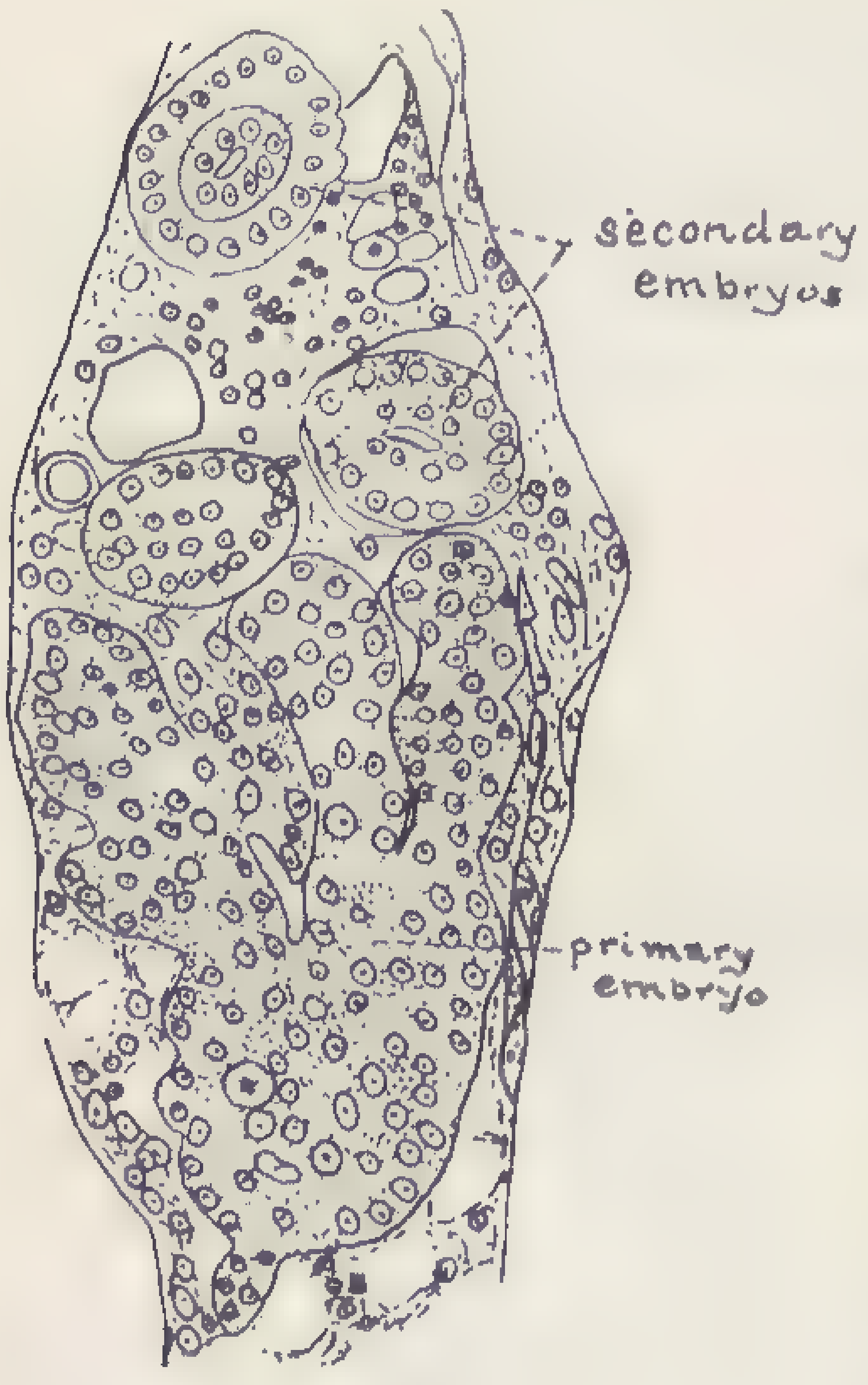

FIG. 29.

5. Under the first head we may include the process of *constriction,* by which two more or less independent masses arise from one. An illustration of this is found in the case of embryonic fission described for some Bryozoa by Harmer (Figure 29). Closely allied to this is the process of sloughing off of a part of the body in metamorphosis, one of the most striking instances of which is shown in

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Fig. 29. Section through a brood chamber of Crisea eburnea, showing the constricting off of secondary embryos from the primary embryo. After Harmer, '93, Quart. Jour. Mic. Sci., XXXIV., Plate XXIII. Fig. 11.

Sacculina, where the thorax and abdomen are thrown off, the head alone persisting to complete the metamorphosis.

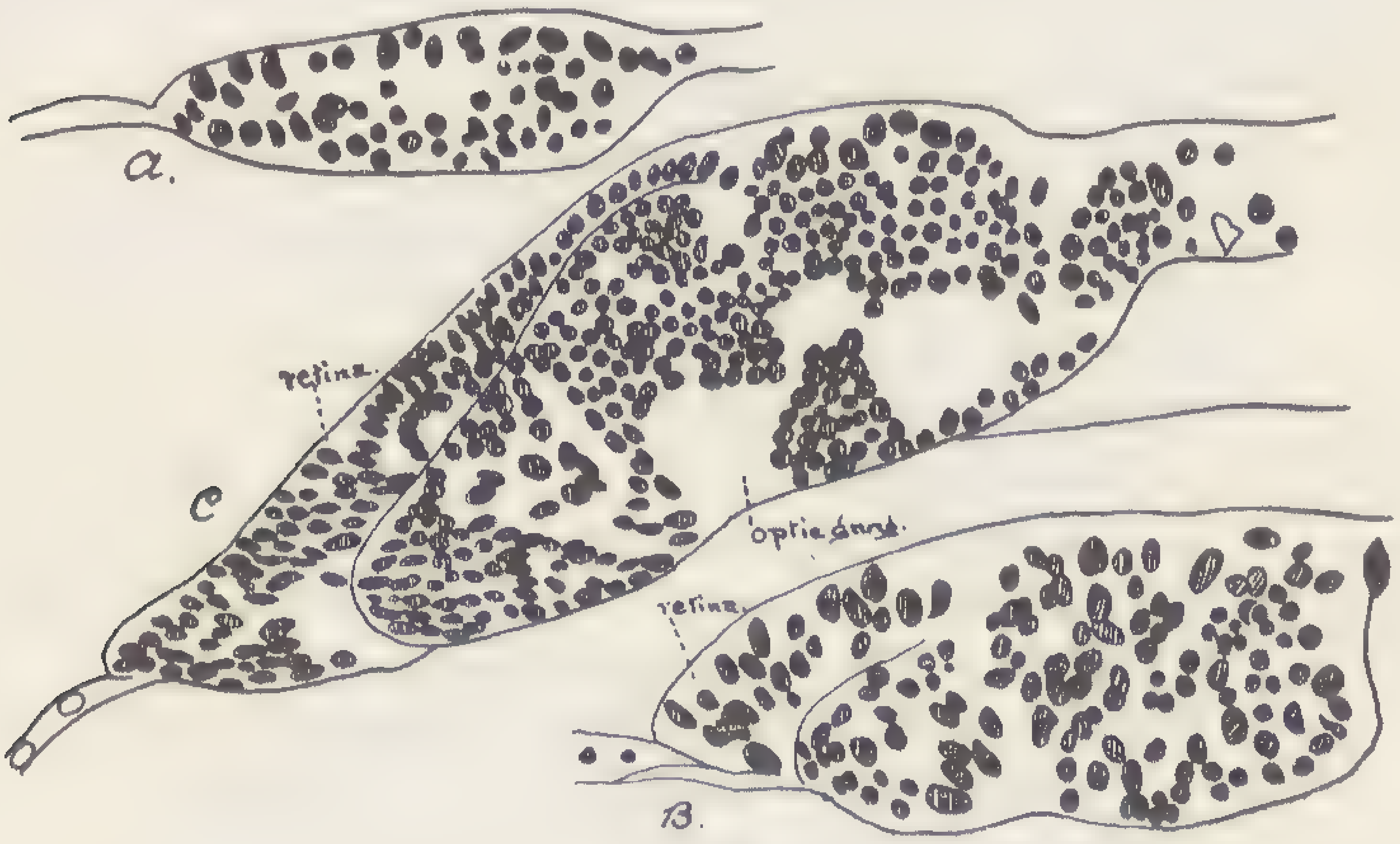

Fig. 30.

6. Another division process is that of *splitting* of the mass. This is illustrated by the case of the optic mass of the lobster (Figure 30), which splits into an outer and an inner part. Compare the origin of the nervous system of Peripatus, K. & H., Fig. 442, B.

7. Under the second head, *fusion* of contiguous masses, we may place such cases as that of the union of independently arisen ganglionic masses, such as Morgan describes for the Pantopod, Pallene (Figure 31).

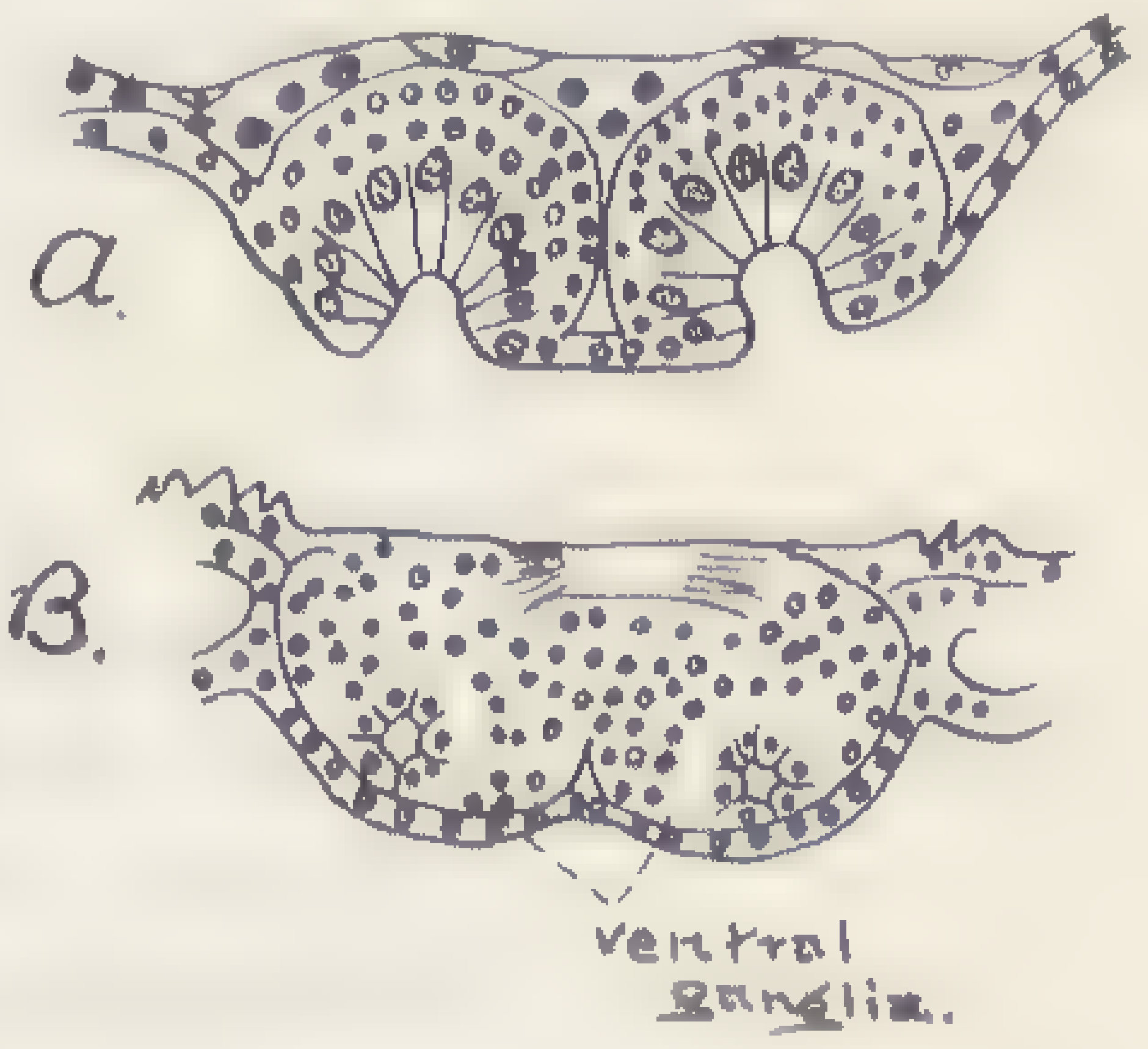

Fig. 31.

Fig. 30. Sections through three stages in the development of the compound eye of the lobster after G. H. Parker. In *B* and *C* the mass is seen to be splitting into the retina and optic ganglion. See K. & H., Fig. 263.

Fig. 31. Ventral part of sections across Pallene embryos. *A*, earlier stage, showing the paired neural invaginations; *B*, later stage, ganglia fused. See K. & H., Figs. 409, 410.

Recapitulation and General Remarks.

We may now, in recapitulation, arrange in tabular form the differential processes which we have recognized.

I. Processes occurring in *mesenchyme.*
  1. Migration of nodal thickenings, p. 175.
  2. Free migration of amœboid bodies, p. 176.
    a. from a layer.
    b. through fluid-filled spaces.
  3. Aggregation of mesenchyme, p. 176.
    a. into a thread.
    b. into a layer.
    c. into a mass.
  4. Attachment of mesenchyme, p. 178.
  5. Investment and interpenetration, p. 178.
  6. Transportation, p. 179.
  7. Absorption, p. 180.

II. Processes occurring in protoplasmic *threads* or *tubules.*
  1. Tropism, p. 181.
  2. Splitting, p. 182.
  3. Anastomosing, p. 183.
  4. Union with other organs, p. 183.

III. Processes occurring in protoplasmic *layers.*
  III^a. Processes affecting *area.*
    α. Processes occurring in the wall of a sac.
      1. Excessive growth of particular parts, p. 184.
        a. along one axis.
        b. at one pole.
        c. along one meridian.
    β. Processes occurring in a plane or warped surface.
      2. Formation of perpendicular folds, p. 185.
        a. Pocket folds.
        b. Linear folds.
      3. Formation of folds in the plane of the area, p. 188.
  III^b. Processes affecting *thickness.*
      4. Thickening, — general or local, p. 188.
      5. Thinning, — general or local, p. 189.
  III^c. Processes affecting *continuity.*
      6. Atrophy, p. 190.
      7. Detachment of a piece from a layer, p. 190.

- III^d. Processes affecting *two or more layers.*
  - 8. Concrescence, p. 191.
    - *a.* of free edges.
    - *b.* of surfaces.
    - *c.* of edges of folds.
  - 9. Perforation, p. 192.
- IV. Processes occurring in protoplasmic *masses.*
  - IV^a. Effecting especially change of *volume.*
    - 1. Excessive growth of particular parts, p. 192.
      - *b.* general.
      - *c.* local.
  - IV^b. Effecting especially change of *form.*
    - 2. Rearrangement of nuclei, p. 193.
    - 3. Vacuolization, p. 194.
    - 4. Dispersion of elements, p. 194.
  - IV^c. Effecting especially change in *number.*
    - 5. Constriction, p. 194.
    - 6. Splitting, p. 195.
    - 7. Fusion, p. 195.

The processes here ennumerated may be for the most part grouped under three general heads: —

- I. Taxic processes.
- II. Tropic processes.
- III. General growth processes.

Under *Taxic Processes* I include such as are accompanied by free migration of protoplasmic bodies, or by the flowing of protoplasm from one part of the whole body to another part.

Under *Tropic Processes* I include such differential growth processes as result in a turning of protoplasmic bodies (threads or folds) towards one another or towards another protoplasmic body.

By *General Growth Processes* I mean those differential growth processes which are not included under tropic processes.

To the category "taxic processes" may be provisionally assigned Nos. I. 1, 2, 3, 4, 5, and 6; III. 4 (in part) and 5 (in part), 7 (?), 9; IV. 2, 4, 5, 6, 7.

To the category "tropic processes" may be assigned Nos. II. 1, 2, 3, 4; III. 8, 9.

To the category "general growth processes" may be assigned Nos. III. 1, 2, 3, 4 (in part) and 5 (in part), 6; IV. 1 and 3.

It will be noted that No. I. 7 (absorption by mesenchyme) is not assigned to any one of the three categories; and that certain other processes (III. 7, Detachment from a layer; II. 2, Splitting; III. 4 and 5, thickening and thinning of a layer; and IV. 3, Vacuolization) are of so doubtful a nature that, although assigned to the special categories, this assignment can be regarded as provisional only.

The process of absorption does not readily fall into one of the three categories, and at the same time it does not seem worth while to erect a special category for it.

As for the doubtful cases, the doubt is not whether they are referable to one of these categories, but rather in knowing in which one to place them.

Regarding the three general categories, it has long been recognized that taxic and tropic processes are responses to stimuli. It has not been so generally recognized that all growth processes are such. A moment's consideration will, however, make this probable.

Let us consider for a moment what it is that controls differential growth, — What makes one part of a membrane grow faster than another, causing a folding of that part?

Inequality of growth is clearly not due to inequality of food supplied, since folds arise in uniformly nourished membranes, — bathed, that is to say, uniformly by the nutritive fluids. It must therefore be due to inequality of the activities which lead to growth; namely, the taking in of food and its assimilation, and the imbibition of water. Now it is our fundamental assumption that activities of all sorts, including ingestion and imbibition, are responses to stimuli. In so far, then, as differential growth is dependent upon the inequality of these activities in different parts of the membrane, it is dependent upon stimuli acting upon that membrane.

Whenever the activities are diverse in the different parts of a membrane, it must be either that the stimulus applied to the different parts is diverse, or, if not, that the protoplasm is diverse in its different parts, for what the result shall be depends upon two factors, — the quality of the stimulus and that of the protoplasm.

Let us now consider somewhat more in detail the taxic and tropic processes. As is well known, the stimuli which control these movements result either in migration towards the source of the stimulus or away from it, so that positive or negative taxis or tropism occurs. In ontogeny it is often impossible to say where the source of stimulation is, and therefore whether the tactic or tropic movements are + or —. Certain criteria may, however, be employed in some cases to determine this. Thus, where many migratory bodies move towards a common point, or where a thread or tubule makes its way to a distant point, we may believe that a positively tropic stimulus is exercised by that point. Also, where two similar parts move towards each other, it is probable that a + stimulus is exerted by both; where, on the other hand, they mutually withdraw, it is probable that a mutual negative stimulus emanates from both.

With these criteria in mind we may classify some of the taxic and tropic processes as + or —, and this I have attempted to do in the following table: —

PROBABLE RESPONSES TO POSITIVE STIMULI.	PROBABLE RESPONSES TO NEGATIVE STIMULI.
Taxic.	*Taxic.*
I. 3. Aggregation of mesenchyme.	I. 2, *a*. Migration from a layer.
I. 4. Attachment of mesenchyme.	III. 4. Thinning of a layer (when due to flowing of the protoplasm from a point).
I. 5. Investment and interpenetration.	III. 9. Perforation.
I. 6. Transportation.	IV. 4. Dispersal of elements.
III. 4. Thickening of a layer (when due to flowing towards one point).	IV. 5, 6. Separation of masses.
IV. 7. Fusion of masses.	
Tropic.	
II. 1. Turning of thread.	
II. 3. Anastomosing.	
II. 4. Union with other organs.	
III. 8. Concrescence.	

PROBABLE RESPONSES TO EITHER + OR — STIMULI.

I. 1. Migration of nodal thickenings.
I. 2, *b*. Free migration of amœboid bodies.
IV. 2. Rearrangement of nuclei.

It is not too much to believe that the foregoing hypothetical interpretation of the ontogenetic processes lies within the possibility of experimental test. Just as the control of the migration of amœboid bodies in the adult has been undertaken with success, so may we hope to control the tropic and aggregation phenomena of ontogeny. By *experiment* alone can the causes of the developmental processes be determined.

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THE EARLY EMBRYOLOGY OF CIONA INTESTINALIS, FLEMMING (L.).

BY W. E. CASTLE.

WITH THIRTEEN PLATES.

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I. INTRODUCTION.

So long ago as 1866, Kowalevsky wrote, "Die Entwicklungsgeschichte der Ascidien wurde schon vielfach studirt." If this statement was true then, it is doubly so now, for the literature of the subject has since that time multiplied many fold. Nevertheless there still remain many unsettled questions regarding the embryology of the Tunicates. Concerning so fundamental a point as the derivation of the primary germ layers in the embryo, quite contradictory opinions have been expressed within the last ten years by observers of world-wide reputation.

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. LII.

I undertook the inquiry, the results of which are recorded in the following pages, in the hope of being able to throw light on this disputed question by the study of other forms than those which had been most carefully examined, and by the application of new methods to the problem. A short experience convinced me that the only method which could yield positive conclusions was that of cell lineage, a method which has been applied so successfully to the study of annelid and molluscan embryology by a number of observers, and had already been employed to a limited extent in the study of ascidian embryology by Van Beneden et Julin ('84), Seeliger ('85), and Chabry ('87).

It soon became clear to me that some of the conflicting statements made by my predecessors arose from errors on their part due to incorrect orientation of certain stages. The nature of these errors I have fully explained in a preliminary communication (Castle, '94). A further study of the embryonic history, cell by cell, through the periods of cleavage and gastrulation, and even down to the differentiation of the several larval organs, has led me to conclusions somewhat at variance with those of earlier investigators regarding the origin of the primary germ layers and the organs derived from them. One of the most important of these conclusions is that the mesoderm of Ascidians — and probably also that of Amphioxus and the Vertebrates — is derived in part from the primary entoderm and in part from the primary ectoderm. The grounds on which this conclusion rests are set forth in the later portions of this paper; in the earlier part of the paper I have recorded some observations on the maturation and fertilization of the ascidian egg.

It gives me pleasure to acknowledge in this place my very great obligations to Professor E. L. Mark for direction and kindly criticism of my entire work. My best thanks are also due to Dr. Alexander Agassiz, in whose laboratory at Newport the material for my studies was chiefly collected, and to Colonel Marshall McDonald for numerous courtesies extended to me at the United States Fish Commission Station at Wood's Holl.

II. MATERIAL, LIFE HISTORY.

The material for this study was collected in the months of August and September of two successive seasons, 1893 and 1894. The species employed seems to be, beyond question, the Ciona intestinalis of Flemming, a classical object of study on the other side of the Atlantic. It was made the subject of an extensive monograph by Roule ('84); its larval history has been studied by Kowalevsky ('66 and '71) and by Willey ('93); its cleavage stages by Samassa ('94); its fertilization stages by Boveri ('90); and the formation of its egg envelopes by Fol ('84). Loeb ('91) also has employed it in certain physiological investigations. The specimens which I collected at Newport answer fully to Roule's detailed descriptions of the species. The large size (8–10 cm. long) attained by individuals at Newport under favorable conditions confirms Roule's conjecture that the forms described from the United States as Ascidia ocellata by Louis Agassiz, as A. tenella by Stimpson ('52), and as Ciona tenella by Verrill ('71) were only small-sized individuals of Ciona intestinalis.

Specimens were obtained by me from two different localities just within the entrance of Narragansett Bay. The animals were usually found adhering to the under side of stones at a depth of from a few inches to a few feet below low-water mark. Upon removal to the laboratory they were carefully washed and placed in aquaria whose water was kept fresh by a jet of air. Once a day the water was changed, and the aquaria thoroughly cleaned, to prevent the accumulation of bacteria or other possibly injurious organisms. This painstaking treatment was probably unnecessary, for the animals are very hardy and bear ill-treatment well. For example, I have kept specimens for weeks at a time in small glass aquaria without change of water, and the only signs of misuse which they exhibited were a slight shrinkage in size and a greatly diminished production of eggs, — both symptoms referable to an insufficient food supply.

Ciona, like all other Tunicates, is hermaphroditic, and the number of eggs produced by a single adult individual in the course of a season must be enormous. Often hundreds are deposited in a single night. Under normal conditions each adult individual, during the summer months, lays eggs once in every twenty-four hours, with the regularity of the sunrise.

Korschelt u. Heider ('93, p. 1267) state that in most cases among the Ascidians self-fertilization appears to be prevented by the ripening of the male and female sexual elements at different periods; although in some cases, where the sexual products mature simultaneously, self-fertilization is not excluded. Neither of these statements holds good for Ciona. Although in the adult period it produces both sexual elements throughout the spawning season, and discharges them simultaneously, self-fertilization rarely occurs, — a conclusion to which I have been led by repeated experiments. The most complete series of these experiments will be briefly described.

The observation had been made that an individual accidentally left overnight in an aquarium by itself laid eggs which failed to develop; whereas, when two or more individuals were placed together in an aquarium, all other conditions being the same, the eggs laid developed almost without exception.

First Experiment.

Acting on the suggestion thus offered, I placed together in an aquarium two or three small, clear individuals (evidently young); in another aquarium was placed an equal number of very large-sized (old) individuals. A greater number of eggs was laid by the large individuals, as one would naturally expect, but the eggs in both aquaria were perfectly fertile. This experiment showed that ripe eggs and sperm are produced both by young and by old individuals.

Second Experiment.

Twenty rather large-sized individuals were selected for experimentation and divided into two lots, A and B, of ten individuals each. The animals of each lot were carefully washed and placed in clean glass dishes filled with fresh sea-water. The individuals of lot A (Table I.) were placed each in a separate dish, those of lot B (Table II.) were placed two in a dish. The next morning a careful examination of each aquarium was made to determine what proportion of the eggs laid had been fertilized. The experiment was repeated on five successive days; on the sixth day, as a control experiment, the lots were interchanged, the animals of lot A (Table II.) being paired, and those of lot B (Table I.) isolated. The results for the six days are embodied in Tables I. and II.

Taking an average of the fifty-eight cases in which eggs were laid by isolated individuals (Table I.), we find that 4.8% of the eggs were fertilized. The occurrence on a single day in two cases of fertilization of 90% of the eggs laid makes me suspect that the dishes were not properly cleaned on that day, and that live spermatozoa may have remained clinging to the sides of the dish after the previous day's experiment. If so, and if

TABLE I. — Close Fertilization.

	Lot A.					Lot B.	
Day.	1st.	2d.	3d.	4th.	5th.	6th.	Total of Cases.
90% fertilized			2				2
25% fertilized	1	1					2
10% fertilized		1	1		1		3
5% fertilized	1	1		1			3
4% fertilized				1			1
None fertilized	8	7	7	7	8	10	47
No eggs laid				1	1		[2]
Total							58 [60]

Average fertility = 4.8%.

TABLE II. — Cross Fertilization.

	Lot B.					Lot A.	
Day.	1st.	2d.	3d.	4th.	5th.	6th.	Total of Cases.
100% fertilized	5	4	4	5	5	5	28
20% fertilized			1				1
None fertilized		1					1
Total							30

Average fertility = 94%.

the dishes were by chance interchanged, a certain amount of cross fertilization may of course have been possible.

On the three succeeding days (fourth, fifth, and sixth) greater precautions were taken, and the jars were dried as well as washed before the experiment was repeated. It will be observed that the proportion of eggs fertilized on those days was distinctly less than on the first three days.

Taking an average of the thirty cases in which eggs were laid in aquaria containing each *two* individuals (Table II.), we find that 94% of the eggs laid were fertilized.

The single instance in which none of the eggs laid in one aquarium were fertilized may be explained by a failure on the part of one of the two animals confined together to emit the sexual products on that particular occasion. Table I. indicates that such cases sometimes occur; for in two instances out of sixty no eggs at all were laid.

Comparing the results of the two tables, we see that under conditions allowing of only close (self-) fertilization (Table I.), less than 5% of the eggs developed; whereas under conditions permitting of cross fertilization (Table II.) at least 90% of the eggs developed.

The question now arose, Do eggs laid by isolated individuals fail to develop because the parent does not discharge sperm at the proper time (perhaps for want of stimulation by another individual), or do the eggs fail to develop because they are *incapable* of fertilization by sperm from the same parent? To settle this point if possible, resort was had to artificial fertilization.

Third Experiment.

The same animals employed in the second experiment were also used in this one. Half of the individuals of each lot were taken for an attempt at close fertilization, the other half being reserved for an attempt at cross fertilization. Each animal was dipped in 90% alcohol to kill any spermatozoa which might be adhering to it; the fingers and instruments used were treated in the same way. Eggs and sperm were removed from the sexual ducts of the animal, and thoroughly mixed in a dish of clean fresh sea-water, the dish having been previously carefully washed and then dried.

The second ten were treated in exactly the same way, except that the sexual products — both male and female — of *two* individuals were mixed together in a single dish.

The proportion of fertilized eggs in each dish was subsequently carefully observed. The results are given in Tables III. and IV.

TABLE III. — Artificial Close Fertilization.

	Cases.
50 % fertilized	1
4 % "	2
1 % "	1
⅛ % "	2
None "	4
Total	10

Average for ten cases, 6% = proportion of eggs fertilized.

TABLE IV. — Artificial Cross Fertilization.

	Cases.
100 % fertilized	4
50 % "	1
Total	5

Average for five cases, 90% = proportion of eggs fertilized.

As the animals employed in the above experiment had been confined in the laboratory for some days, and the production of the sexual elements had in consequence considerably diminished, it was thought desirable to repeat the experiment on animals freshly collected. This accordingly was done with the following equally conclusive results.

TABLE III. *a.* — Artificial Close Fertilization.

	Cases.
50% fertilized	1
12½% "	2
10% "	1
5% "	1
2% "	2
None "	3
Total	10

Average for ten cases, 9.4% = proportion of eggs fertilized.

TABLE IV. *a.* — Artificial Cross Fertilization.

100% fertilized in every case!

The proportion of close fertilized eggs was greater in this experiment than in the preceding. Many of the eggs so fertilized, however, never developed beyond the 2- or 4-cell stage. The cross fertilized eggs all developed normally and at the same rate.

Combining the results of Tables III. and III. *a*, and those of IV. and IV. *a*, we get an average of 7.7% of the eggs developing after close fertilization, and 95% developing after cross fertilization. These averages agree fairly well with those obtained from Experiment 2, which were 4.8% and 94% respectively.

Experiment 3 shows conclusively that, in the case of Ciona, eggs are to a large extent incapable of fertilization by sperm from the same individual as the eggs. Cross fertilization must, therefore, be the rule, and close fertilization the exception under natural conditions. The rare occurrence of close fertilization is probably due to a lack of mutual attraction between eggs and sperm produced by the same individual, an attraction invariably existing between the eggs of one individual and the sperm of another, and probably chemical in its nature. This case is paralleled in certain flowering plants, whose pollen will not germinate when placed on the stigma of the flower from which it was taken, though on the stigma of other flowers of the same species of plant it germinates readily.

There seems to be a particular time of day in the case of each species of simple Ascidian for the discharge of the sexual products. Different aquaria, in which are placed individuals of the same species, if they are subjected to the same conditions of temperature, etc., invariably contain eggs in exactly the same stage of development. This shows conclusively that the time of egg-laying has been the same in the case of each aquarium. For, on account of the rapidity of development, a slight difference in the time of egg-laying would be readily detected by a difference in the stage of development exhibited by the eggs in different aquaria. In the case of Ciona the sexual products are discharged about an hour or an hour and a half before sunrise. The stimulus to their discharge is probably the increasing light of daybreak.

If at about the time mentioned one approaches the aquarium with a lighted lamp, he will see the animals suddenly contract violently two or three times in succession, then resume their accustomed tranquillity. A careful examination will then reveal the eggs floating as little golden specks in the thoroughly agitated water. Soon they begin to settle to the bottom of the aquarium and can then be collected in convenient quantities by means of a pipette. The violent expulsion of the contents of the atrium simultaneously with the release of the sexual products from their respective ducts, must secure under natural conditions a wider distribution and more thorough mixing of the eggs and spermatozoa than would otherwise occur.[1]

The season of spawning of Ciona probably extends in this country, as in Europe, from spring to autumn. I have never collected adult specimens which did not contain mature eggs and spermatozoa, though I have taken them as early in the season as the 10th of June and as late as the 22d of September.

The development of the ovum is very rapid, as I shall show further on, and the larval period brief. The growth of the metamorphosed individual must also be very rapid, as the following facts indicate. In the summer of 1892 specimens of Ciona were abundant in a certain locality at Newport. But the succeeding winter was a cold one, and seems to have killed off those individuals which were situated in very shallow water. In the summer of 1893 specimens were to be found only at a depth of over two feet below low-water mark. In August and September of the next year, however, they occurred in abundance just below low-water mark. But those so situated were rather small, not exceeding 7 cm. in length, very clear, and free from dirt or parasitic growths, thus giving evident signs of youthfulness. They cannot have been over fifteen months old, and may have been much younger. Yet they were sexually mature, and produced eggs in abundance.

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[1] The time of egg-laying is about the same — viz. just before daybreak — in the case of Molgula Manhattensis, on which I made some observations in the United States Fish Commission Laboratory at Wood's Holl, Mass., in June and July, 1894. Cynthia, whose habits I studied at the same place, lays its eggs with equal clock-like regularity, but toward nightfall instead of at daybreak. The late afternoon is also the time of spawning for Amphioxus (Wilson '93, Willey '94). The *manner* of egg-laying is the same in Molgula as in Ciona. Herein my observations differ from those of Kingsley ('83), who states that in Molgula fertilization occurs within the atrium, and that the eggs are for some time afterward retained there. I have never found embryos within the atrial chamber, though I have often seen them adhering to the bodies of the parent individuals, where some eggs had probably settled at the time of spawning. My observations regarding the manner of cleavage in M. Manhattensis also differ from those of Professor Kingsley. He states that the cleavage is unequal, much as in certain Mollusks, and results in the formation of a cap of very small micromeres resting on a few very large macromeres. According to repeated observations of my own, made both on naturally and on artificially fertilized eggs, the cleavage progresses very much as in other Ascidians, the first two cleavages being equal. I think Professor Kingsley must have been misled by appearances in immature eggs obtained by dissecting out the ovaries for artificial fertilization.

Allusion has been made to the rapidity of development of the egg. Within twelve hours after fertilization the larval form is attained, the tail being coiled round the trunk within the egg membranes. Hatching usually occurs within the next twelve hours, i. e. in the first night after the laying of the eggs. It is brought about by twitchings of the larval tail, which finally rupture the egg membranes. Under certain conditions the larva does not succeed in breaking through the egg membranes. Metamorphosis then sets in almost immediately, and is completed within the egg membranes, a functionally free-swimming stage being wholly suppressed. This is regularly the case in Molgula Manhattensis, where hatching of the larva is exceptional, the new, metamorphosed individual arising just where the egg settled after it was thrown out into the water and fertilized. However, in Ciona the more primitive course of events is usually pursued. The larva then escapes from the egg membranes as a miniature tadpole, the "test cells" clinging to its thin and adherent covering of homogeneous, non-cellular mantle substance secreted by the ectoderm. These test cells are soon brushed off as the tadpole swims about; they have no connection, as is now well known, with the cells to be found later in the mantle of the adult.

The larvæ avoid the daylight and swim toward the least brightly illuminated side of the aquarium.[1] Here they attach themselves, usually near the surface of the water, to the side of the aquarium. Sometimes the attachment is by the head end, as it is commonly said to be, but I have more often observed the larvæ attached by the sticky mantle substance at the tip of the tail, the body then hanging head downward against the side of the aquarium.

The larval stage varies in duration from twenty-four hours to several days. It is terminated by the beginning of metamorphosis, whose successive steps are well known through the description of Kowalevsky ('66 and '92), Willey ('93), and others.

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[1] I have observed that the larvæ of Amarœcium also avoid the daylight, i. e. are negatively phototactic; but the larvæ of Botryllus are strongly *positively* phototactic, swarming *toward* ordinary daylight. This difference may perhaps be explained by the difference in habitat of the parent organisms. Botryllus, whose larvæ seek the light, is commonly found in well illuminated places, e. g. adhering to floating eel-grass. On the other hand, Ciona and Amarœcium, whose larvæ avoid the light, more often occur in darkened places, the former on the under side of stones, the latter adhering to piles underneath wharves, or on the sea bottom in sheltered spots near shore.

III. METHODS.

1. Killing, Preservation.

Whenever it was desired to kill a lot of eggs, a sufficient quantity of them was collected in a pipette from the bottom of an aquarium and transferred to a watch-glass, or directly to a small vial of two drams' capacity, in which the eggs were ultimately stored. After the eggs had settled to the bottom of the dish, the water was carefully removed and the killing reagent applied.

The eggs were ultimately preserved in 90% alcohol, and the vials tightly corked, or preferably stoppered with cotton plugs and stored in tightly sealing glass jars. When the latter method is employed, the jars must be kept right side up in transportation, otherwise the small eggs will settle into the cotton plugs and be lost. However, the extra trouble which this method necessitates is well worth taking, for it entirely avoids the injurious effects on preserved material sometimes caused by the tannin which alcohol will extract from corks, if they are used.

Several killing reagents were employed, viz. Flemming's fluid, Hermann's fluid, picro-nitric, corrosive-acetic, and Perenyi's fluid.[1] The blackening effects of the first two reagents made material killed in them unfit for use in the study of eggs as whole objects. Likewise in the case of sections the results from them were disappointing. The only real service rendered by either of these two reagents was in demonstrating in the egg by their blackening effects the character and distribution of the fatty yolk granules. Most serviceable of all the reagents employed on the eggs and embryos up to the period of hatching was Perenyi's fluid. It renders the abundant yolk clear and transparent, and preserves all structures perfectly, without distortion by either swelling or shrinking. Its use does not in my experience interfere in the least with sharp differential staining. The fluid was allowed to act for about twenty minutes, then followed by 70% alcohol, which, to insure removal of every trace of the killing reagent, was changed once or twice in the course of the next twenty-four hours, and replaced at the end of that time with 90% alcohol. A longer treatment with the killing reagent, extending to three or four hours, seemed to give no added advantage, but to interfere slightly with subsequent staining.

Picro-nitric also gave good results, but for the pre-larval stages not so good as Perenyi's fluid, its clearing effects being less. It seems, however, to have been for the larval stages the best reagent which I employed. Davidoff's corrosive-acetic mixture, which has been much used of late by workers on ascidian embryology, is in my experience less faithful in its preservation than Perenyi's fluid, for it shows a tendency to swell certain structures, and lacks the instantaneous hardening effects of that reagent.

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[1] For the composition of the killing reagents and stains mentioned in this paper, see Lee's "The Microtomist's Vade Mecum," 3d edition, London, 1893.

2. Decortication, Staining, Mounting.

The egg of Ciona is surrounded by a series of egg membranes, a correct idea of which is given by the figure of the mature egg of Ascidia canina, reproduced after Kupffer ('72) in Korschelt u. Heider's "Lehrbuch d. vergl. Entwicklungsgeschichte," Figure 736. The egg cell is seen to be surrounded by a clear space — probably occupied by jelly — bounded by the test cells, which are arranged in a rather compact layer one cell deep, so that they seem almost to form an epithelium underneath the chorion. The chorion is a structureless transparent membrane, upon which, as on a basement membrane, the follicle cells ("Schaumzellen") rest. In the egg of Ciona, after it is thrown out into the water, these highly vacuolated cells are even more conspicuous than in the egg of Ascidia as figured by Kupffer. They extend out radially about twice as far as indicated by Kupffer's figure, forming a sort of halo round the egg. The highly refractive nuclei are carried out to the pointed outer ends of the tapering follicle cells.

The presence of the follicle cells and test cells did not interfere seriously with the study of the early stages of cleavage in the living egg, since the clear space between the egg cell and the layer of test cells allows one, with a sufficiently strong illumination, to make out perfectly the outline of the blastomeres and sometimes even nuclear figures in them. But upon preservation in alcohol the envelope formed by the test cells, chorion, and follicle cells collapses, obliterating the clear space and becoming closely applied against the egg cell, thus forming a very serious obstacle to the study of the egg as a whole object. This obstacle I was able to remove by following in a modified form a very ingenious method devised by Chabry ('87, p. 169) for the removal of the follicle cells from the living egg of Ascidiella, a process which he called "decortication." It consisted in simply sucking the eggs into a fine capillary glass tube too small to admit the eggs without the removal of their follicle cells, yet large enough to allow the passage uninjured of the egg itself.

In applying this method to preserved material, I first stained the eggs, as a rule, so that they might be more easily seen. Upon transferring them to alcohol of a low grade, or to water, the egg envelopes would again stand out clear of the ovum, as in the living egg. By then sucking the eggs one at a time into a glass tube of the proper calibre, the entire envelope, consisting of follicle cells, test cells, and chorion, could be removed with considerable facility, and in the majority of cases without injury to the egg itself. Eggs thus decorticated and then mounted afforded excellent surface views.

The eggs are rather opaque, on account of the large amount of yolk which they contain, so that any stain except a very faint one is an obstacle in the study of whole preparations. Excellent results were obtained by mounting in balsam, without any staining whatever, eggs which had been killed in Perenyi's fluid and decorticated.[1] But for the 64-cell and later stages staining was found desirable. Many carmine and hæmatoxylin stains were tried; the one which gave by far the best results being Orth's picro-carminate of lithium. The eggs were treated with a small amount of this stain in a watch-glass for from six to twenty-four hours, then washed thoroughly in water. By this method resting nuclei are stained bright rose-color, while all other structures take only a faint yellow color from the picric acid, and give up even this if the washing is sufficiently prolonged. But the carmine stain in the nuclei is extremely tenacious, and does not fade in the least upon prolonged washing in water or preservation for months in strong alcohol. After the eggs had been stained and decorticated, they were dehydrated, cleared in xylol or cedar oil, then mounted in balsam, the cover glass being supported with small glass rollers made from fine capillary tubes. These served the double purpose of preventing the crushing of the egg and allowing it to be rolled into any desired position by movement of the cover glass. Changing the position of the egg, however, is not often necessary, for at an early stage it takes on a flattened form, which causes it to come to rest with the dorsal or the ventral surface uppermost. This is the case at all periods between the 24-cell stage and that at which the neural tube begins to close, except for a brief period, when the embryo consists of from forty-six to sixty-four cells, and the vertical axis becomes equal to or even greater than the longitudinal axis. Then there is no single position of stable repose for the embryo, and rolling is often necessary to bring it into the positions desired.

The self-orientation of the egg during most of the early stages was of great service in sectioning. When this was desired, the egg, previously studied as a whole object, was returned to xylol. The transfer was accomplished by placing the slide on which it was mounted in a shallow porcelain dish containing a little xylol. This soon dissolved away the balsam, and left the egg free and clearly visible against the white background. The egg was next removed to a shallow watch-glass with a perfectly flat bottom, which was previously smeared with a thin layer of glycerine. Any superfluous xylol was removed from about the egg with filter paper, and a small amount of melted paraffine poured over it, enough to fill the watch-glass to a depth of 3 to 5 mm. The whole was then set over the paraffine bath for fifteen or twenty minutes, when it was placed floating on a dish of water to cool. This being accomplished, the paraffine block was removed from the watch-glass, and the egg, which of course had settled to the bottom and lay with its long axis parallel to the surface of the block, was oriented under the compound microscope in any manner desired. The thinness of the block generally allowed plenty of light to pass through it for this purpose, and it was usually not difficult, owing to the shape of the embryo, to determine its axes. Sections were usually cut $6\frac{2}{3}$ μ in thickness.

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[1] I find that Lillie ('95) has obtained good results in the case of the eggs of the mollusk Unio by mounting, without staining, material killed in Perenyi's fluid. He, however, used glycerine instead of balsam as a mounting medium.

The staining which was found most advantageous for the study of the egg as a whole object was altogether too faint for sections. These were accordingly given a further staining after fixation to the slide. Ehrlich's hæmatoxylin was employed, diluted one half with water. After immersion in the stain for from twenty minutes to an hour, the sections were washed in water to remove the superfluous stain, then to decolorize were placed in 35% alcohol containing 0.1% hydrochloric acid. Here they were allowed to remain until quite pale in color, usually for about five minutes. They were then rinsed in 35% alcohol and held for an instant over the unstoppered mouth of an ammonia bottle, a treatment which gave the hæmatoxylin remaining in the sections a deep blue color, and insured the permanency of the stain. The sections were then passed through the grades of alcohol, cleared in xylol, and mounted in balsam. This process, when properly conducted, resulted in a beautiful and sharply differential double stain. The nuclei retained the light rose tint given them by the carminate of lithium, for the superadded hæmatoxylin stain had been entirely removed from them, except in the chromatic elements, which possessed a deep black color. Cell boundaries, attraction spheres, and other cytoplasmic structures, were clearly brought out, and the fundaments of various organs, as, for example, chorda, mesoderm, and definitive endoderm, were distinguished one from another with great sharpness by the different tints of blue which they exhibited. Iron hæmatoxylin was sometimes employed instead of Ehrlich's, but the results were no better — indeed not so good — for the differentiation of organs or their fundaments.

For studying the processes of maturation and fertilization sections alone could be employed on account of the opacity of the eggs. In making sections of these stages orientation was of course impossible, so that a large number of the eggs was embedded together, without previous decortication, and cut at random. The egg membranes, so far from being an obstacle, were at these stages a positive advantage, since they served to protect and hold the polar globules in place. The material employed in the study of maturation and fertilization stages was killed either in Perenyi's or in Hermann's fluid, the best results being obtained from the former. For convenience the killing of each day will be referred to as a series (A, B, or C), made up of lots (1, 2, 3, etc.) which were killed at intervals of about ten minutes, the first lot being killed as soon after the laying as a sufficient number of eggs could be collected, usually about five or ten minutes.

IV MATURATION AND FERTILIZATION.

The eggs of series A, lot 1, show an early stage in the process of maturation, namely, the formation of the first polar globule. Figure 1 represents a section through one of the eggs of this lot most advanced in development. The egg envelopes, which rest close down upon the egg, are left out in this and all the other figures. Already at this stage we recognize that the egg is made up of two unlike hemispheres, one richer in yolk, the other richer in protoplasm. The former occupies the future *dorsal* or *endodermal* side of the egg, and at the centre of its surface, as stated in my preliminary communication ('94), the polar globules form. The cell division which will give rise to the first polar globule is seen in this figure to be already well advanced, the chromatin being accumulated at the two ends of the spindle. About the deeper end of the spindle there is a small space free from yolk granules and occupied by a finely granular deeply staining mass of protoplasm, of which we shall have more to say. The entire remainder of the dorsal hemisphere, except that small portion of it occupied by the spindle itself, is filled with rounded yolk granules (cf. Fig. 2) of a rather uniform size, closely packed together, but with slender films of staining protoplasm passing between and around them. Davidoff's ('89) beautiful figures, particularly his Tafel VI. Fig. 33, give a correct idea of this "Schaumwerk" structure, if one imagines the yolk granules many times smaller and the protoplasmic films much more slender than in the egg of Distaplia as represented by Davidoff.

The ventral hemisphere also is filled with yolk granules, but here the protoplasmic packing between them is more abundant and less uniformly distributed. It is most conspicuous at the surface, where it forms a thin layer nearly free from yolk granules spreading over almost the whole hemisphere. Within this layer it fades away gradually, but often, as in the case figured (Fig. 1), again becomes prominent at a little deeper level as a series of irregular blotches among the yolk granules; then it once more grows fainter toward the centre of the egg, attaining the condition described for the dorsal hemisphere.

The presence of a spermatozoön cannot be detected in the eggs of this lot. In those of Series B, lot 3, however, its influence is clearly visible. (See Figs. 2 and 4.) About fifteen minutes is estimated to have elapsed between the stage just discussed and the one here presented. At this stage we see in the ventral hemisphere, at some point just beneath the surface, a spherical region entirely free from yolk granules. (See Fig. 2.) Its central portion is occupied by a finely granular substance, which stains in hæmatoxylin an intense blue, shading off somewhat gradually into the more faintly and lightly colored protoplasm occupying the outer portion of the area and continuous with the similarly stained films of the Schaumwerk. At one point the yolk-free region extends out to the surface of the egg. This probably represents the place of entrance of the spermatozoön, which we have reason to believe produces the clear area. The deeply staining substance at the centre of this area is the *male archoplasm* or attraction sphere. It is undoubtedly similar in nature, as it is in optical appearance, to the darkly stained substance seen at the deep end of the maturation spindle in Figure 1, and which may therefore be called the *female archoplasm*. The male pronucleus cannot be made out in the egg a portion of which is shown in Figure 2. In other eggs of the same lot, however, it can be clearly seen; for example, in Figure 4, which represents a stage a little more advanced than the one seen in Figure 2. The area free from yolk is seen in Figure 4 to have enlarged somewhat; the attractive influence of the archoplasm at its centre has manifestly been extended over the greater portion of the hemisphere in which it lies. This fact is indicated *diagrammatically* by the dotted lines in the figure. They are meant simply to indicate that those films of the protoplasmic Schaumwerk which run radially with reference to the attraction sphere have become thicker and more prominent than those running in other directions. Along them as radii doubtless protoplasm is passing to augment the yolk-free area. Nothing in the nature of "fibres" has been observed in them. Excentrically situated in the yolk-free area (Fig. 4) is seen the male pronucleus, a perfectly clear oval body, with a delicate but sharp boundary. Its long axis lies radially with reference to the attraction sphere, which manifestly exerts on it a directive influence. Figure 4 represents the eleventh of a series of eighteen sections. The sixteenth section of the series is shown in Figure 3. It contains the second maturation spindle, at either end of which is an attraction sphere in the centre of a slight accumulation of protoplasm. The chromosomes cannot be clearly made out, but perhaps lie aggregated in a small dark mass close down against the attraction spheres. It is evident that the amount of chromatin involved in this division is less than in the case of the first maturation division (cf. Fig. 1). The obliquity of the plane of sectioning to the dorso-ventral axis of the egg makes this spindle appear to lie quite a little below the surface of the egg. Such, however, is not the case; it comes close up to the surface, but obliquely, not vertically, as did the first maturation spindle. Indeed, an examination of other specimens, less advanced, shows that it first appears in a horizontal position, i. e. at right angles to the direction of the first maturation spindle as seen in Figure 1, but later rotates so that one end of the spindle lies deeper in the egg than the other.[1] The first polar globule does not really lie in this section, but has been projected there from its real position on the margin of the next section, the seventeenth of the series.

In Figure 5 is represented a section, the fifth of a series of sixteen, through an egg of Series A, lot 3, killed twenty minutes later than lot 1 of the same series (cf. Fig. 1). The section passes obliquely in a dorso-ventral direction, unlike those shown in Figures 2–4, which were more nearly horizontal. On the ventral margin of the section is seen the cap of protoplasm which as early at least as the beginning of maturation covered that side of the egg. The male archoplasm has moved deeper into the egg, and its attractive influence has been extended so that it is now manifested over the greater portion of the section. In consequence of this attraction on the protoplasm the area free from yolk has considerably enlarged. The male pronucleus has also increased in size and followed the lead of its attraction sphere toward the centre of the egg. In the dorsal half of the section is seen the female pronucleus, already grown to considerable size. In it can be discerned small chromatic granules, and behind it and deeper in the section the female archoplasm. This archoplasm seems to be much less energetic than that of the male element, for its influence is scarcely perceptible, even on the portion of the egg in which it lies, and it does not appear to modify either the shape or course of the female pronucleus, which, as we shall see, moves toward the male archoplasm leaving its own behind. The polar globules represented at the margin of this section do not as a matter of fact occur in that position, but at the margin of the preceding section. If that section were properly superposed on this, the polar globules would lie over, but a little to the left of the female pronucleus.

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[1] A rotation of the maturation spindles from an original tangential to a radial position has been observed repeatedly in other animals; in the case of the second spindle, the tangential position is doubtless correlated with the derivation of its two archoplasmic masses from the single archoplasmic mass left in the egg after the completion of the first maturation division.

A stage somewhat later than the one just described, though found in the same lot of eggs, is shown in Plate II. Figures 7–10, which represent the fourth, seventh, tenth, and twelfth sections respectively of a series of sixteen. In Figure 7 is seen the male pronucleus with its archoplasm now divided; in Figure 8, the female pronucleus; in Figure 9, the female archoplasm; and in Figure 10, the polar globules marking both the centre of the future dorsal surface of the embryo, and the point from which the female pronucleus starts in its journey through the egg toward the male pronucleus. The position of these various bodies with relation to one another can be most clearly illustrated by two reconstructions (Figs. 11 and 12) upon planes perpendicular to the plane of sectioning and at right angles to each other. Suppose the sections piled one above another in their original order and position, the first section of the series being uppermost and the egg thus reconstructed to be viewed as a transparent object in the direction of the arrow at the left of Figure 7. One would then see the appearance shown in Figure 11, which is a projection of the egg and the most important bodies in it upon a plane parallel to the line *a b* (Fig. 7), and perpendicular to the plane of Figure 7.

If the egg be viewed in the direction of the arrow at the top of Figure 7, one gets the appearance shown in Figure 12, which is a projection upon a plane parallel with the line *a′ b′* in Figure 7, and perpendicular to the plane of that figure.

A comparison of the stage under discussion with that represented in Figure 5 shows that considerable changes have occurred in the interval between them. The male pronucleus (Fig. 7) has grown to much greater size and contains several conspicuous chromatic granules. Instead of a single attraction sphere, there are two, both well defined and at a considerable distance apart. An examination of other eggs of the same lot shows how the condition here existing has come about. The male archoplasm moving in advance of its pronucleus (cf. Fig. 5) has gradually elongated transversely to its line of progress, arranged itself about two centres instead of one, and finally constricted itself into two distinct spherical masses, which move apart, and by their combined action on the male pronucleus draw it forward to a position midway between them, so that its long axis lies in the line joining their centres. The female pronucleus (Fig. 8) has approached to within a short distance (about one fifth the diameter of the egg) of the male pronucleus.

It has grown to an equal size with the male pronucleus, and, like it, contains large chromatic granules. No trace of an archoplasmic body can be seen in connection with it, nor in either of the adjacent sections. However, what are unmistakably the remains of one are visible three sections behind the female pronucleus. (See Fig. 9; compare also Figs. 11 and 12.) This archoplasmic body shows signs of disintegration, being rather diffuse and exerting apparently no attractive influence on the egg protoplasm. The female pronucleus has clearly passed beyond its control, and is now advancing rapidly to unite with the male pronucleus. One might doubt that the body described is identical with a female archoplasm, were it not perfectly constant in its appearance at this stage *behind the female pronucleus in the path of the latter from the point where the polar globules were formed toward the male pronucleus.* Moreover, though diligent search has been made, a similar body has never been found at this stage in any other portion of the ovum, except in connection with the male pronucleus.

In from five to ten minutes after the stage just described the two pronuclei are seen to have come together (Plate III. Fig. 13, and Plate I. Fig. 6). They are indistinguishable from each other so far as size and optical appearance are concerned, and are flattened against each other, but their nuclear membranes remain intact, and there is no mingling of their substance until the first cleavage is about to take place. (See Plate III. Fig. 14.) At the stage shown in Plate II. Fig. 7, we saw that the male pronucleus was already elongated between its two attraction spheres. The female pronucleus is seen in Figure 13 (Plate III.) to have joined it while it is still in that condition. Both have further increased in size. Very soon the nuclear membranes disappear, the attraction spheres move farther apart (cf. Figs. 13 and 14, Plate III.), and a spindle forms between them, on whose equator are seen the chromosomes.

To recapitulate. *In the impregnated egg of Ciona two archoplasmic masses can be recognized, one in connection with each of the pronuclei. That derived from the spermatazoön is much the more energetic of the two, and is alone concerned in bringing the pronuclei together. While the pronuclei are still a considerable distance apart, the male archoplasm divides into two distinct attraction spheres, between which the first cleavage spindle later forms. The female archoplasm degenerates, taking no part whatever in the formation of the first cleavage spindle. There is accordingly in the fertilization of Ciona no union of male and female archoplasms.*

Let us compare briefly these conclusions with those of other recent observers on the subject of the attraction sphere in fertilization.

The fertilization of the Tunicate egg has been studied hitherto by Boveri ('90) and Julin ('93). Boveri's observations, made on Ciona intestinalis and Ascidia mentula, were, as he states, incomplete on account of an accident to his preserved material. It was his opinion that no astral radiations ("Polstrahlungen") are present in the maturation of the egg, and that the two asters of the first cleavage spindle are derived by division from a single one arising in connection with the spermatazoön soon after its entrance into the egg. Julin was able to confirm on Styelopsis grossularia the observations of Boveri, and to supplement them, as he says, by demonstrating at the centre of each aster of the first cleavage spindle a centrosome. No figures, however, accompany Julin's paper; moreover, he states that his observations were restricted to two stages, corresponding to those shown in Boveri's Tafel XII. Figs. 27 and 29.

Though my own conclusions are in entire agreement with those of Boveri and Julin as to the derivation of the attraction spheres of the first cleavage spindle exclusively from the spermatazoön, my observations differ from theirs regarding certain minor points, as the reader may learn by consulting the papers cited.

On the subject of fertilization in groups of animals other than the Tunicata there is an enormous literature. I shall refer to only a few of the most recent papers.

In 1891 Fol described the famous "quadrille of the centres" as occurring in the fertilization of the sea-urchin egg. According to his account, there arises in the egg from the tip of the spermatazoön, a centre of attraction ("spermocentre"), which later divides. In connection with the egg nucleus appears another centre of attraction ("ovocentre"), which likewise divides. Upon the meeting of the pronuclei, each half-spermocentre unites with a half-ovocentre to form an astrocentre. The two astrocentres arise on opposite sides of the cleavage nucleus, and between them the first cleavage spindle forms.

A short time after the publication of Fol's paper, Guignard ('91) described as occurring in the fertilization of a flowering plant a similar union of male and female centres of attraction ("sphères directrices"). More recently Conklin ('94) has observed its occurrence in the case of a mollusk, Crepidula.

Fol's observations, however, are flatly contradicted by the careful studies of Wilson and Mathews ('95) on three different genera of Echinoderms. They find that "the central archoplasm sphere ('attraction sphere') of the cleavage amphiaster is derived by direct and unbroken descent from the central mass of the sperm-aster without visible participation of an egg-aster."

Fick ('93) also observed that in the fertilization of a Vertebrate, Axolotyl, the centrosomes of the first cleavage spindle are derived exclusively from the spermatozoön. Brauer ('92) arrived at a similar conclusion regarding the fertilization of a crustacean, Branchipus, and Mead ('95) regarding a worm, Chætopteris. These observations are in entire agreement with those made prior to Fol's announcement of the "Quadrille" by Boveri ('88) on Ascaris and Sagitta, and by Vejdovsky ('88) on Rhynchelmis. Boehm ('88) had also expressed with some caution a similar view regarding Petromyzon.

On the other hand, Wheeler ('95), in a paper published simultaneously both with that of Wilson and Mathews and with that of Mead, states that in Myzostoma both centres of attraction arise in connection with the *egg nucleus*, none whatever being produced by the spermatozoön.

Summary on Maturation and Fertilization.

(1) *In a majority of the animals in which fertilization has been most recently studied the attraction centres of the first cleavage spindle are derived from the spermatozoön and from the spermatozoön only.*

(2) But *in the fertilization of at least one animal, and undoubtedly in all cases of parthenogenetic development, the attraction centres arise solely in connection with the egg nucleus.*

(3) *Both these facts prove conclusively that the archoplasm, or "organ of division," is not a bearer of heredity,* since in fertilization it may be derived from the sexual product of one parent only, whereas it is a well recognized law that heritable substance is contributed to the offspring by *both* parents equally.

(4) If the archoplasm is furnished in some cases by the sperm only

and in others by the ovum only, it is not inconceivable that in yet other cases *both* may contribute to its formation. Therefore the observations of Guignard and Conklin are not necessarily irreconcilable with those more recently made by Wilson and Mathews, Mead, Wheeler, and myself, as well as the earlier observations of others. In any case, however, the theoretical conclusions based on Fol's "quadrille," as to the share which the attraction centres enjoy in the phenomena of heredity, may now be definitely set aside.[1]

V. POLARITY OF THE EGG.

Attention has already been called to the fact that even before fertilization one axis of the egg, the vertical, has been determined. The point where the polar globules form is its dorsal pole, which lies at the centre of the surface of the *less richly protoplasmic* hemisphere. At some point on the surface of the opposite hemisphere, the spermatazoön usually enters the egg, and there is reason to believe that its point of entrance determines the median plane of the embryo, and so its antero-posterior axis.

After the two pronuclei have met, they move toward the centre of the egg, and in that region the first cleavage spindle arises (Plate III. Fig. 14). It invariably lies parallel to a tangent at the point of formation of the polar globules. The first cleavage plane, which in accordance with a general law is perpendicular to the spindle at its equator, passes through the point where the polar globules arose and divides the egg into two equal blastomeres (Plate III. Fig. 15; cf. Plate V. Fig. 27).

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[1] Boveri ('95), in a paper recently received, completely confirms the observations of Wilson and Mathews regarding the source of the attractive bodies of the first cleavage spindle of the sea-urchin egg. He for the first time in his published writings, so far as I know, gives a formal definition of the centrosome, applying the term to what Wilson and Mathews call the "archoplasm." Boveri, if I rightly understand him, recognizes an archoplasm surrounding the centrosome, at least at certain stages, and specifically different both from the centrosome and from the general cytoplasm.

What in the foregoing pages I have called indifferently *archoplasm* and *attraction sphere* undoubtedly corresponds with what Boveri in his latest paper ('95) defines as the centrosome. A centrosome in the sense of Heidenhain, that is, a simple, distinct granule staining black in iron-hæmatoxylin, I have not been able to detect in the egg of Ciona; nor have I observed a substance (Boveri's archoplasm) specifically distinct from the egg cytoplasm, enveloping the attractive body (Boveri's centrosome). As the reader will glean from the earlier pages of this chapter, I regard the substance forming the radiations about the attractive body as identical with the egg cytoplasm. — June, 1895.

The section seen in Figure 15 shows that cleavage has progressed more rapidly from the ventral than from the dorsal surface. This is to be explained by the richer supply of protoplasm on the ventral surface.

A study by reconstruction or otherwise of a series of sections through an egg in this stage *invariably* shows that cleavage has also progressed with unequal rapidity from the two *ends* of the embryo.

That end at which cleavage is more advanced is destined to become the posterior end. In this case also the inequality in rate of cleavage is attended (probably caused) by an inequality in the distribution of protoplasm. The protoplasmic cap of the ventral hemisphere is always thicker at the future posterior end of the embryo than at the anterior end, and as the first cleavage plane cuts the egg, this accumulation of protoplasm migrates in between the two blastomeres, its presence probably being the accelerating force in the separation of the blastomeres.

After the first cleavage is completed, the protoplasm, which had migrated in between the blastomeres, again returns to the surface and takes up a very definite position on the adjacent faces of the blastomeres just below the equator of the egg. (See Plate III. Fig. 17, *x*.)

This region appears in the living egg as a clear area, and marks the spot where arise later the small flattened posterior cells found so useful in orientation by Van Beneden et Julin and others. That this clear area *is* the region of their formation I have been able to establish by continuous observations of the living egg, controlled and completely supported by the study of preparations. *The thickened spot in the protoplasmic cap of the ventral hemisphere at the beginning of cleavage*, which seems to determine the posterior end of the embryo, *I believe to be caused by the entrance of the spermatozoön*. It is evident that the spermatazoön, unless it enters exactly at the ventral pole of the vertical axis, must lie upon entrance nearer to one end of the egg than to the other, supposing that it is in the median plane and ventral hemisphere of the embryo.

The *nearer* end, I believe, becomes the posterior end of the embryo, and is determined for that fate by the accumulation of protoplasm in the region of entrance of the spermatazoön. It is impossible to say in any particular case exactly where the spermatazoön has entered the egg, for its presence there cannot be detected until it has begun to form a yolk-free area in the egg. However, I have never observed a case in which the spermatozoön did not give evidence from its position of having entered the egg excentrically with reference to the lower pole of the vertical axis. Hence I conclude that cases of entrance *at* that pole, if they occur, are extremely rare.

Summary on Polarity of the Egg.

(1) *The dorso-ventral axis of the embryo is predetermined in the egg before fertilization; the polar globules invariably form at its dorsal pole.*

(2) *The spermatozoön may enter the egg at any point on its ventral hemisphere, that point probably determining, however, the median plane and posterior end of the embryo.*

(3) If we adopt the commonly employed terms *animal* and *vegetative* for the two poles of the unfertilized egg, we must call the ventral the animal pole, and the dorsal the vegetative pole. For it is the *ventral* half of the egg which contains a richer supply of protoplasm, and which consequently cleaves more rapidly and becomes the ectodermal side of the embryo; whereas the dorsal half of the egg contains less protoplasm, cleaves less rapidly, and forms the endodermal portion of the embryo.

(4) We may say, accordingly, that *the form changes accompanying maturation occur, in Ciona at least, and presumably in Ascidians in general, at the pole of the egg opposite to that at which they occur in Amphioxus,* and, so far as known, in all other animals producing eggs with polar differentiation; for the changes connected with maturation are uniformly reported to take place at the *animal,* i. e. at the more richly protoplasmic pole, whereas in Ciona they take place at the vegetative pole.

VI. CELL LINEAGE OF THE EMBRYO.

The statement made in the preceding paragraph presents a condition of affairs so directly contrary to that found in other groups of animals, as well as to what has been assumed by all previous writers to be the case in Ascidians, that it requires the presentation of unmistakable evidence in its support. Such evidence I have to offer, both from the study of the living egg and from that of preparations. Before passing, however, to the consideration of this evidence, a word of explanation is necessary concerning the system of nomenclature to be employed.

1. Nomenclature.

In any extended work on cell lineage it is desirable to have some system of naming the individual cells which will indicate readily the exact history of each, — from what part of the matured ovum it has been derived, by how many divisions it is removed from the ovum, and from what other cells these divisions have separated it. In this paper I shall follow with some modifications the system introduced by Kofoid ('94) in his work on Limax.

1. Each cell will be designated by a letter with two exponents.

2. The letter indicates the quadrant of the egg from which the cell in question has been derived, or in other words that cell of the 4-cell stage from which it is descended. Viewing the egg from the ventral or animal pole (the one *opposite* that at which the polar cells are formed), the left anterior quadrant is A, the right anterior B, the right posterior C, and the left posterior D. In dorsal views, A and D are of course the right quadrants, and B and C the left.

3. The first exponent indicates the *generation* to which a cell belongs; that is, the number of cell divisions by which it is removed from the ovum. The ovum is generation one, the 2-cell stage two, the 4-cell stage three, etc. (See the Table of Cell Lineage on page 275.)

4. The second exponent indicates the *number* of a cell in a generation, the cells of each quadrant being numbered independently from the animal toward the vegetative pole.[1] If in any case two cells of common descent lie in an equatorial position, that one which is nearer the sagittal plane is given the lower numeral.

To ascertain the designation of the mother cell of any particular cell, its *first* exponent must be diminished by one; and its *second* exponent, if an even number, must be divided by two, but if an odd number it must first be increased by one and then divided by two.

In order to determine the daughter cell of a particular cell, simply reverse this process; that is, increase the first exponent by one, and double the second exponent. To determine the other daughter cell, diminish this second exponent by one. For example, the daughter cells of $a^{5\cdot4}$ are $a^{6\cdot8}$ and $a^{6\cdot7}$.

2. Cleavage.

A. Early Stages of Cleavage.

(*a*) *To 24-cell Stage.*

Figures 19–26 (Plate IV.) show eight views of a living egg, drawn by means of an Abbé camera lucida at successive stages, the egg remaining undisturbed in position under the microscope throughout the period of observation. The left side of the egg is, as I shall show, towards the observer. In Figure 19 the process of maturation is seen to be completed, the polar globules lying in a slight depression on the dorsal surface of the egg. The 2-cell stage is shown in Figure 20. The 4-cell stage is seen in Figure 21 to be approaching, and has been reached at the stage shown in Figure 22. The two blastomeres on the side toward the observer appear to be of equal size, the other two are hid from sight. A view of the egg immediately after the next division is shown in Figure 23; the appearance nine minutes later is shown in Figure 24. These both represent the 8-cell stage, and show that *the four cells which lie nearest the polar globules are smaller than those more remote.* They also show that division has occurred in such a manner that the pair of cells occupying the upper right-hand corner of the figure is in contact with the diagonally opposite pair of cells in the lower left-hand corner of the figure, whereas the pair of cells in the upper left-hand corner is entirely separated from that diagonally opposite it. This arrangement is due to no accidental shoving of cells one over another, but is found *invariably* occurring at the 8-cell stage. The diagonally opposite cells which are in contact form respectively the posterior dorsal and anterior ventral portions of the embryo. This arrangement of the cells of the 8-cell stage has up to the present time been overlooked by all writers on tunicate embryology except Chabry ('87). He both distinctly recognized and clearly figured it. (See his Planche XVIII. Fig. 9.) But, as I pointed out in a previous paper ('94), that hemisphere of the egg which he, following Van Beneden et Julin, called *dorsal*, was really the *ventral* hemisphere, so that he wrongly calls the cells in contact the *anterior* dorsal and *posterior* ventral. If we correct his naming of the hemispheres, his observations on Ascidiella are brought into complete agreement with mine on Ciona regarding this point. In both cases the posterior dorsal and anterior ventral cells of the 8-cell stage are in contact. Though Seeliger ('85) apparently overlooked the fact, his figures (Taf. I. Figs. 7, 8, and 10), when their orientation is corrected as I ('94) have shown to be necessary for other reasons, present precisely the same arrangement of cells in the 8-cell stage of Clavelina. This condition is therefore probably of general occurrence among the simple and social Ascidians.

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[1] In gastrulation, the cells about the vegetative pole are depressed to a lower level than the margin of the blastopore. In naming cells it is considered that the vegetative pole is also depressed at that period, and lies constantly on the dorsal surface at the common point of meeting of the cells derived from the four quadrants.

The 16-cell stage immediately after its formation is shown in Plate IV. Fig. 25, and half an hour later in Figure 26. In the stage represented by Figure 26, spindles, directed as indicated by the arrows, were already visible in the large cells, occupying the lower half of the figure, though none had yet appeared in the smaller cells, composing the upper half of the figure. This fact foreshadows an earlier division on the part of the cells of the lower hemisphere, which would lead to a stage of twenty-four cells. Such a stage was figured in my preliminary paper ('94, Plate I. Figs. 1 and 2; here reproduced in Plate IX. Figs. 51 and 52), and it was there demonstrated that the hemisphere in which division is earliest, as the egg passes from the 16-cell stage, becomes later the ventral or ectodermal hemisphere of the embryo.

Accordingly the series of observations illustrated by Figures 19–26 goes to prove that the four larger cells of the 8-cell stage, which are more remote from the polar globules, form the ventral or ectodermal half of the embryo, whereas the four smaller cells, on which the polar globules rest, become the dorsal or endodermal half of the embryo.

The same thing is shown by Figures 27–34 (Plates V. and VI.), a series of drawings of an egg viewed from its anterior end. In Figures 27–29 are seen successive phases of the 2-cell stage. Figure 30 shows the 4-cell stage, and Figures 31 and 32 two phases of the 8-cell stage. At the 8-cell stage in this series, as well as in the series previously examined, the four cells nearest the polar globules are smaller than the other four; they will form, as we shall see, the dorsal hemisphere. There has been no shoving of cells across the median plane, but shoving has occurred among the cells of the right and left halves of the embryo separately, as was seen also at this stage in the series previously examined. (See Plate IV. Fig. 23.) According to the rule already stated, we should find in contact with each other the diagonally opposite pairs of cells which are to form respectively the posterior dorsal and anterior ventral portions of the embryo; while the other two pairs of cells should be completely separated. If this is true in the case before us (Figs. 31 and 32), we are looking at the anterior end of the embryo, for the pair of *ventral* cells *nearest* the observer is seen to be in contact with the most *remote* pair of *dorsal* cells.

Figure 33 (Plate VI.) shows the 16-cell stage, and Figure 34 the 24-cell stage in process of formation. In this egg also the cells of the hemisphere most remote from the polar globules were first to divide in passing from the 16-cell stage. Those of the other hemisphere divided in this case about twelve minutes later. Therefore *by this series also the hemisphere more remote from the polar globules is shown to be the ventral or ectodermal.* That one is looking in this series at the anterior end of the embryo, as already suggested, and not at the posterior end, is shown by a comparison of Figure 34 (Plate VI.) with Figure 51 (Plate IX.), both of which represent the 24-cell stage. The posterior end of the embryo is seen in Figure 51 to be marked by a noticeably small pair of cells, the like of which does not appear in Figure 34, but may be supposed to lie hidden from view at the more remote end of the embryo. Moreover, the cells $A^{6.1}$, $A^{6.2}$, $B^{6.1}$, $B^{6.2}$ of Figure 51, which are situated at the anterior end of the embryo, correspond well in size with the four cells nearest the observer in Figure 34. Therefore the rule previously stated for the orientation of the 8-cell stage is exemplified in this series also.

In Figures 45–50 (Plate VIII.) is shown another series of drawings illustrating what has been said regarding the clear protoplasmic region (x) which throughout cleavage marks the posterior end of the embryo. In this series one looks down obliquely on the dorsal surface of the embryo from its posterior end. The polar globules are not visible, for the reason that they do not come into profile at the margin of the egg, a circumstance which is necessary for an exact determination of their position.

In Figure 45, a 2-cell stage, the clear region appears in each blastomere at x. During each successive cell division it bulges out as represented in Figure 46, and again in Figure 47, just as if it were the most plastic portion of the egg and responded most readily to the internal tension which accompanies cell division. Such indeed is probably the case, for this region is free from yolk granules, consisting of protoplasm only, as has been already pointed out.

In Figure 48, the 8-cell stage is seen to be completely formed. Applying our rule for the orientation of the egg at this stage, we decide that the pair of cells occupying the centre of the figure and nearest to the observer is to form the posterior dorsal portion of the embryo; for (1) it belongs to the set of four smaller cells formed by the first equatorial plane of cleavage, and (2) it is in contact with the diagonally opposite pair of cells of the other hemisphere. The sequel justifies our conclusion. Figure 49 represents the 16-cell stage, and Figure 50 the 24-cell stage.[1] In Figure 50 it is seen that the small posterior cells of the ectodermal hemisphere, unmistakably identical with $C^{6.8}$ and $D^{6.8}$ of Figure 51 (Plate IX.), have appeared just where the clear portions forming prominences at the time of cell division have all the time been. These portions have become a part of the small cells in question, which contain less yolk than any other cells of the egg at this stage, and subsequently cleave less rapidly than any other cells of the ventral hemisphere. The persistence of this clear polar region in stages later than that of 24 cells was shown in certain figures of my preliminary paper (reproduced in Plate IX. Figs. 54 and 55). It finally passes into the small flattened cells $C^{7.5}$, $D^{7.5}$ (Plate XI. Fig. 71), of whose later history we shall have more to say.

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[1] It will be observed that between the stages represented in Figures 49 and 50 there has been a slight rotation of the egg, so that the latter figure exhibits an exactly dorsal view instead of an obliquely dorsal one.

Chabry ('87) observed in Ascidiella at the beginning of the 8-cell stage the formation of polar prominences such as I have described, and spoke of them as a sure means of orienting the egg at this stage. On page 203 he says: "Il est encore une marque propre aux cellules P et P [$D^{4.1}$, $C^{4.1}$] que permet de les distinguer de toutes les autres, elle consiste en une petite saillie en forme de mamelon, saillie qui est dirigée horizontalement en arrière et que montrent les figures 2 et 23 de la planche XVIII. Cette saillie n'est visible qu'au début du stade VIII. [8-cell] et surtout durant la segmentation qui produit P et P [$D^{4.1}$, $C^{4.1}$]." Apparently Chabry overlooked the formation of the prominences at other than the 4- and 8-cell stages, and failed to recognize their true significance. For he explains them as merely foreshadowing the form and direction of the next cell division, and as referable to a supposed general phenomenon, which, stated in his own words, is as follows: "Que les blastomères ont à l'instant où ils viennent de se produire et mieux encore *durant leur individualisation* des formes spécifiques qu'ils perdent peu d'instants après. Ces formes spécifiques paraissent être en rapport avec les segmentations dont ces blastomères seront plus tard le siège . . . la segmentation a donc lieu dans tous les cas, perpendiculairement au plus grand axe que possédait le blastomère durant son individualisation."

It is hardly necessary, I suppose, to say anything at this late day in refutation of Chabry's generalization. My own observations indicate that cells tend to assume at the time of their formation ("individualisation") a *spherical* form,[1] if they are homogeneous in structure, and that the departure from an evenly rounded contour at the posterior end of the ventral hemisphere is explicable by the presence there of a region peculiar in its constitution, containing as it does less yolk than the other superficial portions of the egg.[2]

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[1] Mutual pressure of cells may modify this form, in which case the direction of the next division may perhaps be predicted, as Chabry states, at the time of the "individualisation" of cells. For, *other things being equal*, it is true that the spindle arises in the longest axis of the cell.

[2] I am aware that Van Beneden et Julin ('84) have offered an entirely different explanation for certain phenomena probably related to those under discussion, which they observed in the cleaving egg of Clavelina. Their explanation implies

Let us examine still another series of drawings (Figs. 35–42, Plates VI. and VII.) made from the living egg, which in this case is viewed from the ventral side and a little obliquely. The polar globules of course are not seen, since they lie on the opposite side of the egg. Neither is the point of view a favorable one to bring the posterior polar regions clearly into profile as in the series last examined.

Figure 35 (Plate VI.) shows the 4-cell stage; Figures 36 and 37, successive views of the 8-cell stage; and Figure 38, a 12-cell stage, the four cells of the ventral hemisphere having divided in this case a little earlier than those of the dorsal hemisphere. This is unusual, for the difference in rate of cleavage of the cells of the two hemispheres commonly first appears, as we have seen in the three series previously examined, in passing from the 16-cell stage to one of 24 cells.

Figure 39 (Plate VII.) gives a view of the egg five minutes after the stage shown in Figure 38 had been reached. It represents the 16-cell stage. A drawing made five minutes later still is shown in Figure 40, and one made ten minutes after that is shown in Figure 41.

In the last mentioned figure, the cells of this uppermost hemisphere are seen to have again become rounded in outline preparatory to the next cell division. Spindles are already visible in them, as indicated by the arrows, those last to appear being the ones in the small cells ($C^{5.2}$, $D^{5.2}$) at the lower margin of the figure. The subsequent division was about a minute later in these two cells than in the others of the same hemisphere; this is regularly the case in the cell division which leads to the 24-cell stage.

Figure 42, the last of the series, will be at once recognized, by one who has read my preliminary paper, as a ventral view of the 24-cell stage. (Cf. Plate IX. Fig. 51.) The posterior end is clearly marked by the small cells $C^{6.3}$, $D^{6.3}$. A re-examination of Figures 36 and 37 (Plate VI.) shows that the rule previously stated for orienting the egg at the 8-cell stage is again exemplified in the case of this series, for in

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the existence during karyokinesis of *astral fibres* which attach to the cell wall at particular points and by their contraction depress its surface.

Such an explanation seems to me inadequate, at least for this case; first, because I have seen no evidence of the existence of astral *fibres* in karyokinesis; secondly, because at successive cleavages the prominences appear in the same structurally peculiar region, whether the karyokinetic spindle is directed *toward* that region — as the explanation of Van Beneden et Julin would imply — or not (see Plate VIII. Fig. 47); thirdly, because astral fibres, if present, should appear in every blastomere at karyokinesis, but I have been able to discover these peculiar prominences only in the particular regions already described.

Figures 36 and 37 we see in contact cells which we know, from an examination of Figure 42, eventually become the anterior ventral and posterior dorsal portions of the embryo.

We have now followed the cleavage cell by cell to the 24-cell stage. We have seen that cleavage is from the very beginning bilateral, and progresses in a very definite manner and at a very definite rate. This we shall find is true in the further development of the egg, even until the complete closure of the blastopore. Wilson ('94) observed that the cleavage of Amphioxus showed all gradations between a perfectly radial, a bilateral, and even a spiral form; and he raised a query whether the same might not be found to be true for Ascidians. In Ciona at least this does not seem to be the case. I have never observed an instance of deviation from the regular mode of cleavage described in the foregoing paper, unless one so construes the occasional very slight difference in the time of cleavage of the cells of the two hemispheres in passing from the 8-cell stage, a matter to which allusion was made on page 232. No rotation of the cells of one hemisphere over those of the other even in the slightest degree has ever been observed. In having a perfectly definite and stereotyped manner of cleavage, the ascidian egg resembles more closely the egg of Annelids, Mollusks, and the great majority of Invertebrates, than it does that of Amphioxus and the Vertebrates, notwithstanding that the *end product* of cleavage shows unmistakably the now generally admitted closer affinity of Tunicates with the latter group of animals.

It remains to call attention to some of the internal phenomena accompanying the early cleavage stages. The first cleavage spindle arises, as has been stated, not far from the centre of the egg. (See Plate III. Fig. 14.) As its first cleavage is nearing completion, however, the attraction spheres and nuclei begin to move toward the dorsal surface o the egg, *away from* its more richly protoplasmic (animal) pole, from which the plane of separation cuts in more rapidly. (See Plate III. Fig. 15.) The attraction sphere of each blastomere grows more diffuse as the nuclei pass into a resting condition; it then elongates in a horizontal direction and parallel to the first plane of cleavage, and finally divides. The parts separate and the nucleus moves out to a position between them. (See Plate III. Fig. 16.) By this time the attraction spheres and nuclei unmistakably lie closer to the dorsal (maturation) surface of the egg. (Plate III. Fig. 16; cf. Plate IV. Figs. 20, 21, and Plate V. Figs. 27–29; also Van Beneden et Julin's ['84] Figs. 2 and 4 *b*, Planche VII., remembering that dorsal and ventral are reversed in Van Beneden et Julin's figures.) The yolk-free protoplasm trails downward from the attraction spheres forming a sort of crescent in each blastomere. (See Plate III. Fig. 16.)

During the second and third cleavages the nuclei remain somewhat nearer the dorsal (vegetative) pole. (Plate IV. Figs. 20–22, and Plate V. Fig. 30; cf. Van Beneden et Julin's ['84] Figs. 4 *b* and 5, Planche VII.) It follows naturally that when the 8-cell stage is formed by the first equatorial plane of cleavage (third cleavage), an inequality is observed in the size of the newly formed blastomeres, the four nearer to the *dorsal* pole being *smaller* than their sister cells, though the latter are richer in protoplasm. (See Plate IV. Fig. 23, and Plate III. Fig. 18.)

(*b*) *Summary on Early Cleavage Stages.*

1. The future posterior end of the embryo is marked at the 2-cell stage by an accumulation of protoplasm free from yolk in each blastomere at contiguous regions. This accumulation persists throughout cleavage, and forms at each cell division a pair of protuberances beyond the general contour of the blastomeres.

Subsequently to the 8-cell stage, in each of the two blastomeres in which these accumulations lie, the spindle at three successive cell divisions is directed *toward* the protoplasmic accumulation of that cell and lies *nearer* to it than to the opposite side of the cell. In consequence the newly formed cell, which contains the region in question, is in each case *smaller* than its sister cell. (Cf. $D^{5.1}$ and $D^{5.2}$, Fig. 38, Plate VI.; $D^{6.2}$ and $D^{6.3}$, Fig. 51, Plate IX.; and $D^{7.5}$ and $D^{7.6}$, Fig. 62, Plate X.)

At each of these divisions also cleavage occurs *later* in the cells containing the protoplasmic accumulations than in their sister cells.

2. The first cleavage plane is vertical, and passes through the point of formation of the polar globules. It coincides with the future median plane of the embryo, and divides the egg into two blastomeres equal in size and similar in every particular. They form respectively the right and left halves of the embryo. The fate, as just stated, of the first two blastomeres of the ascidian egg was first pointed out in the case of Clavelina by Van Beneden et Julin ('84).

3. The second cleavage plane is also vertical, and at right angles to the first. Like the first, it passes through the point of formation of the polar globules. It divides the egg into four blastomeres, among which no difference of size can be recognized.

4. The third cleavage plane is at right angles to both the preceding, i. e. equatorial in position. It separates four smaller cells lying nearer to the polar globules and more abundantly supplied with yolk, from four larger ones more remote from the polar globules and richer in protoplasm.

The former are destined to give rise to the dorsal or endodermal hemisphere of the embryo; the latter, to the ventral or ectodermal hemisphere.

The protoplasmic accumulations mentioned under paragragh 1 always fall in the posterior pair of cells of the ventral hemisphere (viz. $D^{4.1}$, $C^{4.1}$) close to its line of contact with the dorsal hemisphere. This pair of cells is never in contact with the anterior pair of cells of the dorsal hemisphere, but the *anterior* pair of cells of the ventral hemisphere (viz. $A^{4.1}$, $B^{4.1}$) is *invariably* in contact with the posterior pair of cells of the dorsal hemisphere (viz. $c^{4.2}$, $d^{4.2}$).

The fact just stated affords a ready and unfailing means of orienting the 8-cell stage. This was recognized by Chabry in the case of Ascidiella, and is shown by an examination of Seeliger's figures to be equally true for Clavelina. It probably holds good among all the simple and social Ascidians.

5. The 16-cell stage is usually reached by simultaneous divisions in both hemispheres. Sometimes, however, the cells of the ventral hemisphere at this cleavage divide sooner than those of the dorsal hemisphere, thus giving rise to a 12-cell stage, but this very soon changes to a 16-cell stage by the cleavage of the cells of the dorsal hemisphere.

6. As the egg passes from the 16-cell stage, cleavage *invariably occurs earlier* in the cells of the ventral hemisphere, i. e. the descendants of the four larger cells of the 8-cell stage, than it does in the cells of the dorsal hemisphere. A 24-cell stage results, in which the cells of the ventral hemisphere, being twice as numerous as those of the dorsal hemisphere, cover more surface and begin the process of overgrowth (epiboly), forcing the cells of the dorsal hemisphere into a somewhat columnar form. (See Plate VII. Fig. 44.)

B. LATER STAGES OF CLEAVAGE.

(*a*) *From 24-cell to 46-cell Stage.*

The 24-cell stage was taken as the point of departure in my preliminary paper ('94), and the cell lineage was traced in detail through a stage of 46 cells. I shall not repeat except in the form of a brief *résumé* what was there said regarding those stages, but shall content myself with reproducing (in Plate IX.) the figures of Plate I. accompanying that paper, which were executed to illustrate this period of the developmental history. These figures present dorsal and ventral views of the 24-cell stage (Plate IX. Figs. 51 and 52), the 32-cell stage (Figs. 53 and 54), and the 46-cell stage (Figs. 55 and 56).

The interpretation, as given in my preliminary paper, of the lineage through the 46-cell stage rested upon the strongest possible evidence, viz. the observation of karyokinetic figures for every cell division which was represented as having occurred. Moreover, it was shown that these observations made it possible to reconcile the conflicting statements of others who had studied the cleavage of the ascidian egg. Such excellent observers as Van Beneden et Julin, on the one hand, and Seeliger, on the other, held contrary opinions as to which was the dorsal side and which the anterior end of the embryo in its early stages in one and the same genus, Clavelina.

It was shown in my paper, both from an examination of the authors' own figures and from a comparison with the lineage of Ciona, that their conflicting statements arose from a fundamental error on the part of each, Van Beneden et Julin being correct in their determination of the *ends* of the embryo, and Seeliger in his determination of the dorsal and ventral *surfaces* of the early stages. Upon correcting these mistakes, it was found that the observations of the writers mentioned were brought into harmony, and were then in agreement with my own observations on Ciona.

In order to demonstrate that I had correctly determined the dorsal and ventral faces of the egg for the 46-cell and earlier stages, in contradiction to the interpretation of Van Beneden et Julin, I figured a single older stage described as one of 66 cells (Castle '94, Plate II. Figs. 11 and 12). Its presentation was intended to bridge the gap between the 46-cell stage and gastrulation. This purpose it fulfilled, for it showed gastrulation already commenced, and so proved beyond question which was to be the oral (dorsal) and which the aboral (ventral) surface.

A desire to give completeness to my figures led me to state the *lineage* of this stage as I then understood it. I have since found, from the study of more complete series of embryos than I had at that time secured, that I was mistaken as to the time of cell division in one pair of cells ($C^{7.8}$, $D^{7.8}$, Fig. 56, Plate IX.). I supposed it had already occurred at the stage represented in Figures 11 and 12 (Plate II.) of my former paper. Consequently the lineage there given for this stage is incorrect. Though this fact does not affect the main conclusions of my preliminary paper, it necessitates modification of several minor statements, as will be indicated in detail later.

The 24-cell stage, it has been seen, arises from the 16-cell stage by an earlier division on the part of the cells of the ventral hemisphere than occurs in those of the dorsal hemisphere. Accordingly, we find that at the 24-cell stage the ventral hemisphere consists of sixteen cells, whereas the dorsal hemisphere is made up of only eight. These eight are compressed into a columnar form by the overgrowth of the cells of the ventral hemisphere already begun. (See Plate VII. Fig. 44.) Their nuclei lie in a superficial position, while their deep ends are heavily laden with unassimilated yolk. They retain this columnar form up to and throughout gastrulation. In number, they are soon brought up to an equality with the cells of the ventral hemisphere by division, which leads to the 32-cell stage (Plate IX. Figs. 53 and 54) and places all the cells of the egg in the sixth generation.

Presently the cells of the ventral hemisphere again anticipate in division those of the dorsal hemisphere, this time by a still longer interval. Among the cells of the ventral hemisphere differences in the time of division could, as we have seen, be detected at the preceding cleavage. At the present cleavage the differences become more pronounced. In particular, the small posterior cells, $C^{6.3}$, $D^{6.3}$ (Plate IX. Figs. 53 and 54), divide enough later than their fellows to allow us to recognize a 46-cell stage (Plate IX. Figs. 55 and 56), made up as follows: —

Ventral hemisphere, 28 cells in the seventh generation,
2 cells ($C^{6.3}$, $D^{6.3}$) in the sixth generation.
Dorsal hemisphere, 16 cells in the sixth generation.
46

When the two small cells $C^{6.3}$, $D^{6.3}$, divide, which they do earlier than the cells of the dorsal hemisphere, a stage of forty-eight cells is reached, all the cells of the ventral hemisphere (thirty-two in number) being in the seventh generation, and those of the dorsal hemisphere (sixteen in number) being in the sixth generation. Such a stage is shown in Plate X. Figs. 57 and 58.

(*b*) *48-cell Stage.*

The embryo shown in Figures 57 and 58 has a vertical axis the length of which is equal to that of its antero-posterior axis, if not greater. Accordingly it has been found easier to maintain this axis in a horizontal position, and hence more convenient to represent the egg as viewed from the anterior and posterior ends respectively, rather than from the dorsal and ventral surfaces, as in most of the other stages figured. This stage (48-cell) is made up as follows: —

Ventral hemisphere, 32 cells in the seventh generation.
Dorsal hemisphere, 16 cells in the sixth generation.
48

It will be observed that the cells of the ventral hemisphere, though all in the seventh generation, are not all equally advanced in their preparations for division, which evidently is again about to set in. For while the cells occupying the centre of the ventral hemisphere, or, in other words, lying nearest to the animal pole of the egg, are about to pass into the next generation, the cells occupying the margin of the ventral hemisphere, and more remote from the animal pole, contain nuclei entirely quiescent, like those seen in the cells of the dorsal hemisphere. This is contrary to the statement made in my preliminary notice ('94), in which I said that at this division those cells of the ectodermal hemisphere which were marginal and in contact with cells of the endodermal hemisphere were *first* to divide. This erroneous statement arose from the wrong interpretation given to Figures 11 and 12 ('94 Plate II.) in describing the cell lineage of that stage, a matter to which attention has already been directed.

In the embryo shown in Plate X. Figs. 57 and 58 (48-cell stage) one may readily distinguish three regions, each composed of sixteen cells. The first region is the dorsal hemisphere, with its sixteen cells all in the sixth generation ($a^{6.5}$–$a^{6.8}$, $d^{6.5}$–$d^{6.8}$, and the corresponding cells in quadrants B and C). These cells are destined to form the endoderm of the larva, the chorda, and a portion of the mesoderm. The second group of sixteen cells occupies the centre of the ventral hemisphere ($A^{7.1}$, $A^{7.2}$, $A^{7.3}$, $A^{7.5}$, $A^{7.7}$, $D^{7.1}$, $D^{7.2}$, and $D^{7.3}$, with the corresponding cells in quadrants B and C). They are in the seventh generation, but already contain spindles, showing that they are soon to pass into the eighth generation. This group of cells will form the ectoderm of the larva. The remaining sixteen cells of this embryo, also belonging to the ventral hemisphere, form the third group ($A^{7.4}$, $A^{7.6}$, $A^{7.8}$, and $D^{7.4}$–$D^{7.8}$, with the corresponding cells in quadrants B and C). They too are in the seventh generation, but their nuclei are quiescent, showing that these cells will be later in dividing than the other cells of the ventral hemisphere. They are arranged in an equatorial band between the other two groups of cells. This band is interrupted at only one point on each side of the embryo, where a single cell ($A^{7.5}$, $B^{7.5}$) of the ectodermal group reaches up into contact with the cells of the dorsal hemisphere. From the equatorial band just described are derived chiefly nerve cells and mesoderm cells.

(*c*) *64-cell Stage.*

The completion of the divisions foreshadowed by spindles in the ectodermal group of cells of the stage last discussed (Plate X. Figs. 57 and 58) doubles the number of cells in that group, and brings the number in the entire embryo up to sixty-four, distributed as follows.

Ventral hemisphere (designated by the letters *A*, *B*, *C*, *D*) : —
32 cells in the 8th generation = the ectodermal group.
16 " 7th " = the equatorial band.
48
Dorsal hemisphere (designated by *a*, *b*, *c*, *d*) : —
16 cells in the 6th generation.
64

Such a stage is shown in Plate X. Figs. 59 and 60, the former representing a ventral and the latter a dorsal view. The egg has again assumed the flattened form which it had at the 32-cell stage.

Examining first the ventral surface (Fig. 59), we see that the divisions foreshadowed in the 48-cell stage (Figs. 57 and 58) have in every instance occurred in a direction perpendicular to that of the spindle in the mother cell, though a slight displacement is in some cases appearing among the daughter cells, on account of the mitoses arising in the equatorial band. The cells of the ectodermal group, on account of their recent division, now number thirty-two, as many as are found in both the other groups put together. They are in the eighth generation, one generation in advance of the cells of the equatorial band, and two generations in advance of the cells of the dorsal hemisphere. They are $A^{8.1}$–$A^{8.6}$, $A^{8.9}$, $A^{8.10}$, $A^{8.13}$, $A^{8.14}$, and $D^{8.1}$–$D^{8.6}$, together with the corresponding cells in quadrants *B* and *C*.

The equatorial band is, as at the last stage, composed of sixteen cells all in the seventh generation, but six of them (three on each side of the median plane, Fig. 60, $A^{7.4}$, $A^{7.8}$, and $D^{7.4}$) now show signs of approaching division. Four of these mitotic cells form the anterior segment of the equatorial band, and are destined to produce a considerable portion of the nervous system of the larva. (See Fig. 60, $A^{7.4}$, $A^{7.8}$, $B^{7.4}$, and $B^{7.8}$.) The two remaining mitotic cells of the equatorial band are situated laterally one in each of the posterior quadrants (Figs. 59 and 60, $D^{7.4}$, $C^{7.4}$).

The ten remaining cells of the equatorial band all contain resting nuclei. Eight of these cells are grouped at the extreme posterior end of the equatorial band in a region where, from the 16-cell stage on, we have found cleavage to be more tardy than in any other part of the ventral hemisphere. These eight cells are $D^{7.5}$, $D^{7.6}$, $D^{7.7}$, $D^{7.8}$, and the corresponding cells in quadrant C. (Figs. 59 and 60. Compare Fig. 57.) The two remaining cells of the equatorial band which still show no signs of division are $A^{7.6}$ and $B^{7.6}$ (Fig. 60), situated about midway between the anterior and posterior ends of the embryo.

Of the sixteen cells comprising the dorsal hemisphere (Fig. 60), six, which lie in contact with the equatorial band ($a^{6.5}$, $a^{6.7}$, $d^{6.6}$, with their mates in quadrants B and C), are in mitosis. Four of them, the most anterior of the cells of the dorsal hemisphere, lie in a transverse row across the dorsal surface of the embryo (Fig. 60, $a^{6.7}$, $a^{6.5}$, $b^{6.5}$, and $b^{6.7}$). They will ultimately form the greater portion of the chorda. We will call them the *anterior chorda fundament.* The two other mitotic cells of the dorsal hemisphere are $d^{6.6}$ and $c^{6.6}$, in the posterior half of the embryo (Fig. 60). The spindles in these cells are directed obliquely forward, upward, and outward, so that, taking into consideration the superficial position of the nuclei of the dorsal hemisphere, we may predict that the coming division will result in cutting off in each case a smaller more superficial cell from a larger cell extending deeper; the small cell will also lie anterior and lateral to its sister cell. The unequal divisions in these two cases will separate cells of unlike fate; the two smaller cells will constitute the *posterior chorda fundament,* the two larger ones will form mesoderm.

The ten remaining cells of the dorsal hemisphere (Fig. 60, $a^{6.6}$, $a^{6.8}$, $d^{6.5}$, $d^{6.7}$, $d^{6.8}$, and the corresponding cells in quadrants B and C) show as yet no signs of division. They are grouped about the vegetative pole of the egg, the point of origin of the polar cells, and *will form the whole of the definitive endoderm of the larva, and nothing else.*

(*d*) *76-cell Stage.*

Upon the completion of division in the twelve mitotic cells of the embryo represented in Plate X. Figs. 59 and 60, a stage of seventy-six cells would be reached. An embryo in this stage is shown in Plate X. Figs. 61 and 62. It contains in the

Ventral hemisphere (designated by the letters *A*, *B*, *C*, *D*):
32 cells in the eighth generation = the ectodermal group.
12 cells in the " " } = the equatorial band.
10 cells in the seventh " }
54 —

Dorsal hemisphere (designated by *a*, *b*, *c*, *d*): —
2 mesoderm cells in the seventh generation.
10 chorda cells in the " "
10 endoderm cells in the sixth "
22 —
—
76

The ectodermal group of the ventral hemisphere contains the same number of cells as at the 64-cell stage, viz. thirty-two, — sixteen on each side of the median plane, ten of them being derived from an anterior quadrant (*A*), six from a posterior quadrant (*D*). They cover nearly the entire ventral surface of the egg. (See Plate X. Fig. 61, $A^{8.1}-A^{8.6}$, $A^{8.9}$, $A^{8.10}$, $A^{8.18}$, $A^{8.14}$, and $D^{8.1}-D^{8.6}$, as well as the corresponding cells in the right half of the figure.) All the cells of this group are in the eighth generation.

The equatorial band now contains six more cells than at the 64-cell stage, in consequence of the completion of divisions foreshadowed at that stage in the cells $A^{7.4}$, $A^{7.8}$, $B^{7.4}$, $B^{7.8}$, $C^{7.4}$, and $D^{7.4}$ (Fig. 60). It now consists of twenty-two cells, which, in passing from the posterior end forward, are $D^{7.5}$, $D^{7.6}$, $D^{7.7}$, $D^{7.8}$, $D^{8.7}$, $D^{8.8}$, $A^{7.6}$, $A^{8.16}$, $A^{8.15}$, $A^{8.8}$, and $A^{8.7}$, with the corresponding cells in quadrants *B* and *C* (Fig. 62). Six of the cells on each side of the median plane are derived from a posterior and five from an anterior quadrant. Signs of approaching division have at this stage become visible in four of the cells of this equatorial band, viz. $A^{7.6}$, $B^{7.6}$, $D^{7.7}$, and $C^{7.7}$. In the case of the first two cells mentioned the spindles stand vertically (cf. Plate X. Fig. 67, $A^{7.6}$); in the other two cells ($C^{7.7}$, $D^{7.7}$) the spindles are nearly horizontal in position, though their antero-lateral ends lie at a slightly higher level than the opposite ends.

There are only six cells remaining in the equatorial band which neither have passed into the eighth generation nor show any signs of immediately doing so. They are grouped at the posterior end of the embryo, which has been repeatedly pointed out as the region of slowest cleavage among the cells of the ventral hemisphere. The six cells in question are $D^{7.5}$, $D^{7.6}$, $D^{7.8}$, and the corresponding cells in quadrant *C* (Fig. 62).

Although for convenience I shall continue to use the term equatorial band, it is clear that the cells composing it are no longer strictly equatorial in position, but now lie on the flattened dorsal surface (Fig. 62). This change of position has come about in consequence of the more rapid cell division in the ventral hemisphere. How considerable the difference in rate of division has been between the cells of the two hemispheres, one readily appreciates if he stops to consider that the cells of the ventral hemisphere now number fifty-four, whereas those of the dorsal hemisphere number only twenty-two.

In the dorsal hemisphere (Fig. 62) the divisions foreshadowed by spindles at the 64-cell stage (Fig. 60) have taken place, but no new ones are approaching. The number of cells in this hemisphere is now twenty-two; twelve of them (the chorda and mesoderm cells, cf. description of Fig. 62 in the explanation of Plate X.) are in the seventh generation, and ten (the endoderm fundament), in the sixth generation, no divisions having occurred in the last named group of cells since the 32-cell stage. Of the ten chorda cells, eight derived from the anterior quadrants are arranged in a crescent-shaped band capping the anterior end of the dorsal hemisphere; they are $a^{7.9}$, $a^{7.10}$, $a^{7.13}$, $a^{7.14}$, and the corresponding cells in quadrant *B*. They form the anterior chorda fundament. The other two chorda cells, which are derived from the posterior quadrants, are $d^{7.11}$ and $c^{7.11}$. They form the posterior chorda fundament, and are at present separated from the anterior chorda cells by two cells of the equatorial band, viz. $A^{7.6}$ and $B^{7.6}$.

The sister cells of $d^{7.11}$ and $c^{7.11}$, viz. $d^{7.12}$ and $c^{7.12}$, are the sole contribution of the dorsal hemisphere to the mesoderm of the larva, for the greater part of the mesoderm is, as we shall see, derived from the equatorial band.

Among the endoderm cells it is noticeable that $d^{6.5}$ and its mate $c^{6.5}$ have been shoved forward out of their own quadrants to a position beside the endoderm cells derived from the anterior quadrants.

(*e*) *Summary on Later Cleavage Stages.*

1. In the cleaving ovum one can recognize, in passing from the animal to the vegetative pole successive zones, in each of which cleavage takes place less rapidly than in the preceding. At the 64-cell stage (Plate X. Figs. 59, 60) there are three such zones: first, a group of thirty-two cells encircling the animal pole, all of them in the *eighth* generation; second, an equatorial zone of sixteen cells, all in the *seventh* generation; third, a group of sixteen cells encircling the vegetative pole,

all in the *sixth* generation. The first two zones are descended from the four ventral cells of the 8-cell stage, i. e. from the four cells most remote from the point of formation of the polar globules. The third zone is descended from the four dorsal cells of the 8-cell stage. The ectoderm is derived chiefly from the first zone, — that is, the zone encircling the animal pole; — the mesoderm is derived chiefly from the second zone, and the endoderm exclusively from the third zone.

This zonal arrangement persists throughout cleavage and the early stages of gastrulation, but its symmetry is at each succeeding stage disturbed to an increasing extent by the fact that cell division is less rapid at the posterior than at the anterior end of the embryo.

2. Although, as just stated, cleavage progresses with unequal rapidity at the two poles of the antero-posterior axis, as well as at those of the dorso-ventral axis, it is *equal* in rate at the two poles of the third axis of the egg, viz. the transverse. The last mentioned fact serves to maintain the perfectly bilateral form of the embryo.

The differentiation of the poles of the dorso-ventral and antero-posterior axes, the reader will recall, was already recognizable by structural cytoplasmic differences in the unsegmented ovum. *The form and rate of cleavage are therefore manifestly predetermined by the internal constitution of the ovum.*

3. Gastrulation.

A. Early Stages of Gastrulation.

(*a*) *112-cell Stage.*

An embryo a little more advanced in development than the one represented in Figures 61 and 62 (Plate X.) is shown in dorsal view in Figure 71 (Plate XI.). No new divisions have occurred in the dorsal hemisphere, which accordingly consists, as at the last stage, of twenty-two cells. In the equatorial band, the four cells which were preparing for division at the 76-cell stage (Plate X. Fig. 62, $D^{7.7}$, $C^{7.7}$, $A^{7.6}$, and $B^{7.6}$) are seen in Figure 71 to have divided, though in the case of $A^{7.6}$ and $B^{7.6}$, on account of the vertical position of the spindles (cf. Fig. 67), only the more superficial daughter cell is in each instance visible ($A^{8.12}$, $B^{8.12}$, Fig. 71). No further divisions have occurred in the equatorial band, which therefore consists at this stage of twenty-six cells, all in the eighth generation except the group of six cells arranged in crescent form at the posterior end of the embryo, viz. $D^{7.5}$, $D^{7.6}$, $D^{7.8}$, and the corresponding cells in quadrant C. These cells have lingered in the seventh generation later than all other cells of the ventral hemisphere.

The equatorial band as a whole has now moved to a position distinctly within the margin of the dorsal surface (Fig. 71), so that a row of cells from the ectodermal group of the ventral hemisphere has come into view outside it round almost the entire periphery of the embryo (*cf.* Fig. 62). This change has come about in consequence of additional divisions in the ectodermal group of cells, which now not only has spread over the entire ventral surface of the embryo, but is encroaching upon its dorsal surface.

Division has occurred nearly synchronously in all the cells of the ectodermal group, though somewhat sooner in those nearest the animal pole. (See Plate X. Figs. 63–70.) The strongest possible confirmation of my own observation regarding the simultaneousness of division in the cells of the ectodermal group in this period of development is afforded by Samassa's ('94) Figures 10 and 11, Taf. II. These represent respectively a dorsal and a lateral view of a stage intermediate between those shown in my Figures 62 and 71. In Samassa's Figures 10 and 11, all the cells of what I have called the equatorial band are figured as containing quiescent nuclei, except the four seen to be mitotic in my Figure 62 (Samassa's cells 3 and 6, Fig. 10). The other cells of the ventral hemisphere visible in Samassa's figures are without exception in process of division. It may accordingly be confidently assumed that at the stage shown in Figure 71 the cells of the ectodermal group of the ventral hemisphere have all passed into the ninth generation. If so, they number sixty-four; this agrees well with the approximate count which one can make from dorsal and ventral views, though it is impossible to be sure about the exact number of ectoderm cells lying at this stage in an equatorial position between the dorsal and ventral surfaces. Accordingly I shall not attempt to give for this and subsequent stages the lineage of the individual cells of the ectodermal group. This would be a work of great difficulty and of some uncertainty, for in this case the cells entirely lack those marked differences of size, stainability, and arrangement which make the lineage for the cells of the equatorial band and dorsal hemisphere a matter of perfect definiteness.

If the estimate given of the number of cells in the ectodermal group is correct, the embryo shown in Plate XI. Fig. 71 represents a stage of one hundred and twelve cells distributed as follows.

Ventral hemisphere: —

64 cells in the 9th generation = the ectodermal group,
20 " 8th " ⎫
6 " 7th " ⎭ = the equatorial band.
90 —

Dorsal hemisphere: —

2 mesoderm cells in the 7th generation.
10 chorda " 7th "
10 endoderm " 6th "
22 —
90 (in ventral hemisphere).
112

The process of gastrulation has at this stage already set in. Not only is the ectoderm growing over so as to envelop the dorsal hemisphere, but the latter is at the same time sinking down and becoming saucer-shaped. (Cf. Figs. 66 and 77.) Accordingly, gastrulation may be said to take place by a combination of the two processes of epiboly and invagination.

(*b*) *Differentiation of the Principal Organs as seen at the 112-cell Stage.*

a. TOPOGRAPHICAL.

We will now consider this same embryo (Plate XI. Fig. 71) with reference to the ultimate fate of its cells. At the depressed centre of its dorsal surface, surrounding the point of formation of the polar globules, we find the ten cells of the definitive endoderm, all in the sixth generation and containing each a very large nucleus. They are $a^{6.6}$, $a^{6.8}$, $d^{6.5}$, $d^{6.8}$, $d^{6.7}$, and the corresponding cells in quadrants *B* and *C*. Two of them are derived from each of the anterior quadrants (*A* and *B*), and three from each of the posterior quadrants (*C* and *D*). Together they constitute the entire fundament of the definitive larval endoderm.

The endoderm fundament is surrounded by two concentric rows of cells from which are derived some of the most important organs of the larva. The inner row or ring of cells we will call the *chorda-mesenchyme ring*, because it is destined to produce the chorda and mesenchyme. In it we must include the small flattened cells, $C^{7.5}$, $D^{7.5}$, but not their sister cells, $C^{7.6}$, $D^{7.6}$, which, though in contact superficially with endoderm cells, really belong, as their fate shows, in the second or outer ring.

The chorda mother cells, all of which are included in the chorda-mesenchyme ring, are derived, as has been already stated, in part from the anterior and in part from the posterior quadrants. Those derived from the anterior quadrants are at this stage eight in number. They form the anterior segment of the chorda-mesenchyme ring (Fig. 71, $a^{7.9}$, $a^{7.10}$, $a^{7.13}$, $a^{7.14}$, and the corresponding cells on the left of the median plane). The posterior chorda cells are only two in number

($c^{7.11}$, $d^{7.11}$, Fig. 71), one in the right and one in the left half of the embryo. They are now separated both from each other and from the anterior chorda cells. We shall see later how they are brought into contact with each other, in the median plane, and with the anterior chorda cells.

The mesenchyme mother cells are also ten in number, but, unlike the chorda cells, they are derived chiefly from the *posterior* quadrants. They are $A^{8.12}$, its deep-lying sister cell, $A^{8.11}$, $d^{7.12}$, $D^{7.8}$, and $D^{7.5}$, with the corresponding cells in the left half of the embryo, all indicated by a flat tint in the Figures.[1] It will be observed that *the mesenchyme fundament is made up of cells derived from both hemispheres and all four quadrants.*

The outer of the two rows of cells encircling the endoderm fundament will be called the *neuro-muscular ring.* (Fig. 71. The cells are stippled.) It is interrupted at three points by mesenchyme cells of the inner ring; in the middle line behind, by the small flattened cells, $C^{7.5}$, $D^{7.5}$; on the right side, by $A^{8.12}$; and on the left side, by $B^{8.12}$. It is thus divided into three portions, an anterior segment of eight cells, all descended from the anterior quadrants, and two latero-posterior segments, each composed of four cells, descended from one of the posterior quadrants. The anterior segment is composed purely of nerve mother-cells, which will form a considerable portion of the medullary plate. The other segments will form the entire longitudinal musculature of the larva, as well as a certain portion of the nervous system in the tail region.

In the two rings of cells just described are included all save two of the descendants of the cells forming the *equatorial band* of the 48-cell and later stages. These two cells are $D^{8.13}$ and $C^{8.13}$, situated at the posterior margin of the embryo (Fig. 71). They form, in my opinion, definitive ectoderm.

The remaining cells of the embryo number sixty-four, all descendants of the *ectodermal group* of the 48-cell stage. They will form definitive ectoderm, possibly also a portion of the medullary plate.

One again notices in this stage the striking difference in rate of division of the cells which he meets in passing from the vegetative toward the animal pole, a difference which made itself apparent as early as the

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[1] Samassa ('94) identified the mesenchyme mother cells $D^{7.8}$ and $d^{7.12}$ (the cells 8 and 9 of his Fig. 10) as nerve cells. In my preliminary paper I expressed a different opinion, stating that they were mesoderm cells. Subsequent study has confirmed this view, but shown that I was wrong in stating, as I did, that they would contribute to the formation of "the longitudinal musculature of the tail." That organ has, as I shall show, an entirely different and hitherto unsuspected origin.

16-cell stage, and was foreshadowed still earlier by the internal constitution of the unsegmented ovum. The endoderm fundament is in the sixth generation[1] (Plate XI. Fig. 71, $d^{6.7}$, $d^{6.8}$, $d^{6.5}$, $a^{6.8}$, $a^{6.6}$, and the corresponding cells in the left half of the Figure); the chorda-mesenchyme ring is chiefly in the seventh generation, though a single pair of its cells has recently passed into the eighth ($D^{7.5}$, $D^{7.8}$, $d^{7.12}$, $d^{7.11}$, $A^{8.12}$, $A^{8.11}$, — the deep-lying sister cell of $A^{8.12}$, not shown in the Figure, — $a^{7.14}$, $a^{7.13}$, $a^{7.10}$, $a^{7.9}$, and the corresponding cells in quadrants B and C); the cells of the neuro-muscular ring are all in the eighth generation, except a single pair which lingers in the seventh ($D^{7.6}$, $D^{8.14}$, $D^{8.7}$, $D^{8.8}$, $A^{8.16}$, $A^{8.15}$, $A^{8.8}$, $A^{8.7}$, and the corresponding cells in quadrants B and C); the ectoderm cells are all in the ninth generation, those nearest the animal pole having been the first to divide and pass into that generation. We notice in this stage, as in the earlier stages, a region of delayed division in the equatorial band at the posterior end of the embryo.

β. Histological.

Figures 63–70 (Plate X.) represent eight cross sections from a series through an embryo (not figured) a little more advanced in development than the one shown in Plate X. Figs. 61 and 62. The approximate position in the embryo of the sections figured is indicated by the horizontal lines on Figure 62.

The differing stainability of cells at this stage, together with other histological peculiarities, serves already to distinguish the fundaments of the various organs with considerable precision.

The endoderm cells ($d^{6.7}$, Fig. 64; $d^{6.8}$, Figs. 65 and 66; $a^{6.8}$, $d^{6.5}$, Fig. 67; $a^{6.6}$, Fig. 68; together with the corresponding cells in the left halves of these Figures) are, on account of their slow division, still very large. They are columnar in form, and contain large nuclei. Their cytoplasmic portion scarcely stains at all except in the region of the nuclei, being almost entirely taken up with close-packed yolk granules. A small amount of protoplasm staining a bright blue in haematoxylin extends out from either side of the nucleus in the long axis of the cell. In this small protoplasmic mass evidently lies an attraction sphere close up to the wall of the nucleus. The nuclei themselves contain [illegible] chromatic granules.

The mesenchyme cells ($D^{7.8}$, Fig. 64; [illegible] Fig. 67; together with the corresponding cells in the left halves of these Figures) are sharply distinguished from those of every other tissue by the very intense blue color which they take upon treatment with a hæmatoxylin stain. Their cytoplasm is not homogeneous at this stage, but contains numerous large dark-looking granules. The granules are undoubtedly yolk granules, and their dark appearance can often be seen upon close inspection to be due to an enveloping film of deeply staining protoplasm, which often extends out in radial processes, giving the whole a star-like appearance. This I believe to be caused by the progressive assimilation of the yolk granules and their conversion into protoplasm. In the case of the mitotic cell $A^{7.6}$ (Fig. 67), and likewise of its mate in the left half of the same Figure, the characteristic mesenchyme staining appears only in the more superficial portion of the cell, its deeper portion being loaded with yolk granules, which are still almost unattacked by the protoplasm. Consequently, when the approaching division is accomplished, the sister cells formed will differ from each other in appearance, the more superficial one being deeply stained, the other being stained scarcely at all. Subsequently, however, the yolk-laden cell will come to resemble in appearance its sister cell, and will have the same ultimate fate. The nuclei of the mesenchyme cells resemble closely in appearance those of the endoderm cells. In the case of $c^{7.12}$ and $d^{7.12}$ (Figs. 65 and 66), the nuclei are relatively small on account of recent division.

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[1] See the table on page 275.

The eight *anterior* chorda cells ($a^{7.14}$, Fig. 68; $a^{7.9}$, $a^{7.10}$, and $a^{7.13}$, Fig. 69; together with the corresponding cells in the left halves of these Figures) resemble closely in shape and stainability the endoderm cells. They are smaller, however, and contain nuclei, likewise smaller, with less conspicuous chromatic granules (omitted altogether in the Figures, as previously explained, to aid in readily distinguishing the chorda cells from those of other organs).

The two *posterior* chorda cells ($d^{7.11}$, Fig. 66; $C^{7.11}$ [by mistake of engraver for $c^{7.11}$], Fig. 67) stain more deeply than the anterior chorda cells, resembling to some extent their sister cells $d^{7.12}$ and $c^{7.12}$ (Figs. 65 and 66), from which they have recently been separated by division. However, they are many times smaller than their sister cells, and extend less deeply. This difference is connected with the oblique position of the spindles in the mother cells (see $d^{6.6}$, Fig. 60), a matter to which attention was called in the discussion of the 64-cell stage.

In the neuro-muscular ring the cells (stippled to distinguish them from those of other groups) have about the same histological character in both anterior ($A^{8.16}$, Fig. 68; $A^{8.15}$, Fig. 69; $A^{8.7}$, $A^{8.8}$, Fig. 70; together with the corresponding cells in the left halves of these Figures) and posterior ($D^{8.8}$, Fig. 65; $D^{8.7}$, Fig. 64; $D^{7.6}$, $D^{8.14}$, Fig. 63; together with the corresponding cells in the left halves of these Figures) portions. The cytoplasm is finely granular and pretty homogeneous throughout, except in those portions of the cell most remote from the nucleus, where a certain amount of yolk is to be seen either unassimilated (Fig. 66, $C^{8.8}$) or in process of assimilation (Figs. 68–70, $A^{8.16}$, $A^{8.15}$, $A^{8.8}$, $A^{8.7}$, and their mates in quadrant *B*). In $C^{7.6}$ (the mate of $D^{7.6}$ in Fig. 63) both conditions are realized. Around the nucleus is the finely granular protoplasm, and in those portions of the cell most remote from the nucleus is the unattacked yolk. Forming a sharp line of boundary between the two is a zone in which assimilation is progressing, the yolk granules appearing here as large dark bodies. The color which the cells of the neuromuscular ring assume is not so deep a blue as that of the mesenchyme cells; it is of a more grayish tint.

B. LATER STAGES OF GASTRULATION.

(*a*) *From the 112-cell to the 128-cell Stage.*

Figure 72 (Plate XI.) represents a dorsal view of a stage a little more advanced than the 112-cell stage shown in Figure 71. Sections (not figured) of this stage show (cf. sections of an older stage, Figs. 73–77) that the endoderm cells are in mitosis, the spindles being in all cases situated in an approximately horizontal position, so that after division the daughter cells will lie in a single layer forming a curved plate. The spindles are directed longitudinally in all the cells except two, viz. $c^{6.8}$ and $d^{6.8}$ (cf. Plate X. Fig. 62), in which they lie transverse to the long axis of the embryo.

Among the mesenchyme cells division has occurred in $D^{7.8}$, $C^{7.8}$ (cf. Figs. 71 and 74), the spindles standing vertically, as in the case of $A^{7.6}$, $B^{7.6}$ (Plate X. Figs. 62 and 67), which divided earlier. Vertical spindles are also present in $c^{7.12}$, $d^{7.12}$ (cf. Plate XI. Figs. 71 and 75).

The chorda cells are in the same generation as at the last stage, but the anterior ones are laterally compressed into a flattened or wedge shape, their thinner edges being directed backward. They are situated at the anterior margin of the blastopore (Fig. 72).

In the neuro-muscular band, two cells on each side of the blastopore ($D^{8.7}$, $D^{8.8}$, $C^{8.7}$, $C^{8.8}$) are seen to be in mitosis, their spindles being directed toward the centre of the blastopore. No evidence of division can be seen in any other cells of the embryo. It is therefore clear that the considerable advance in the process of gastrulation which is seen to have taken place since the stage shown in Plate XI. Fig. 71, has come about chiefly by an invagination, independent of cell division on the aboral surface, which has carried inward the endoderm and mesenchyme cells, has left at the margin of the wide-open blastopore the anterior chorda cells and the muscle cells, and has brought plainly into view, outside the neuro-muscular band, another row of cells from the ectodermal surface extending round the entire margin of the embryo. A certain number of cells at the anterior end of this new ring is destined to serve the same purpose as the anterior segment of the neuro-muscular band; this fact is indicated in the figure by stippling.

A clearer idea of the changes just sketched in outline may perhaps be had from an examination of cross sections. In Figures 73–77 are represented five sections through the region of the blastopore of an embryo a little more advanced than the one shown in Figure 72. The approximate position of the sections in the embryo is indicated by horizontal lines at the margin of Figure 72. In the endoderm cells the long deferred division leading to the seventh generation has at last been accomplished (Figs. 73–77). The endoderm cells accordingly number twenty, and their nuclei are greatly reduced in size on account of the recent division (cf. $d^{6.8}$, Fig. 66, Plate X., with $d^{7.15}$, $d^{7.16}$, Fig. 75, Plate XI.). The columnar form of the mother cells is retained by their descendants.

It has been already stated that the spindles in the endoderm cells were at the recent division approximately horizontal in position. It is evident, therefore, that before the accomplishment of division the attraction spheres must have shifted from the position which they were seen to occupy in Plate X. Figs. 66–68, for otherwise the spindles would have stood vertically, and a two-layered arrangement of the cells would have resulted, such as we shall see does occur in the case of the mesenchyme cells. No mechanical explanation of this change in the position of the attraction spheres in the endoderm cells offers itself. The longest axis of the cells appears to be continuously the vertical axis, yet the spindles form in a direction transverse to this in every instance. Van Beneden et Julin's ('86) Figures 1 *c* and 2 *c* also show spindles occupying the short axis of the endoderm cells in the case of Clavelina.

Considering now the mesenchyme cells we see (Fig. 74) that $D^{\cdot 8}$, $C^{7.8}$ (Fig. 71) have divided in such a manner that a small superficial cell is separated in each case from a many times larger deep-lying sister cell (cf. Fig. 74, $D^{8.16}$, $D^{8.15}$, $C^{8.16}$, $C^{8.15}$). A division similar in direction and in the inequality of its products is foreshadowed for the next anterior pair of mesenchyme cells (Fig. 75, $c^{7.12}$, $d^{7.12}$), in which the spindles lie much nearer to the superficial than to the deep ends of the cells. The most anterior mesenchyme cells appear in Figure 77 ($A^{8.12}$, $A^{8.11}$, $B^{8.12}$, $B^{8.11}$). They are descended from $A^{7.6}$, $B^{7.6}$ (Plate X. Fig. 62), in which cells the division leading to the eighth generation occurred at a stage considerably earlier than this. (See Plate X. Fig. 67.) In this case also ($A^{7.6}$, $B^{7.6}$) the spindles stood vertically and division was unequal, but the *more superficial* daughter cell was the larger, the deeper one being small and almost entirely filled with unassimilated yolk. (See Plate XI. Fig. 77, $A^{8.12}$, $A^{8.11}$, $B^{8.12}$, $B^{8.11}$.)

The posterior chorda cells ($d^{7.11}$, Fig. 77, and $c^{7.11}$ in the left half of Fig. 76, not lettered) show no essential change since the last stage figured (cf. Plate X. Figs. 66 and 67). The anterior chorda cells do not appear in the sections figured; they are still in the seventh generation.

Division has been completed in $D^{8.7}$, $D^{8.8}$, $C^{8.7}$, $C^{8.8}$ (Fig. 72), four of the neuro-muscular cells lying at the margin of the blastopore. (See Figs. 74–76, $D^{9.13}$, $D^{9.14}$, $D^{9.15}$, $D^{9.16}$, and the corresponding cells in quadrant *C*.) The other cells of the neuro-muscular ring and the entire ectodermal group have not been essentially modified since the last stage figured (Fig. 72).

If the foregoing account is correct, the embryo, sections of which are shown in Figures 73–77, contains one hundred and twenty-eight cells, distributed as follows.

Ventral hemisphere :—
  Ectodermal group :
    64 cells in the 9th generation { ectoderm. / nerve cells.
  Equatorial band :
    2 ectoderm cells in the 9th generation.
    8 neuro-muscular cells in the 9th generation.
    10 " " " 8th "
    2 " " " 7th "
    8 mesenchyme " 8th "
    2 mesenchyme " 7th "
  32
96

Dorsal hemisphere :—
    2 mesenchyme cells in the 7th generation.
    10 chorda " " "
    20 endoderm " " "
  32
128

(*b*) *Closure of the Blastopore.*

In Figure 78 (Plate XI.) is shown a section parallel and slightly lateral to the median plane of an embryo a little more advanced than any thus far examined. It shows how the closure of the blastopore is coming about.

The ectoderm cells on the ventral surface are seen to be smaller toward the anterior (left in the figure) than toward the posterior end of the embryo. They have evidently divided and passed into a later generation than those more posteriorly situated. This has caused them to spread over a greater surface, and has shoved the cells anterior to them farther around on to the dorsal surface of the embryo. In the anterior chorda cells, one of which is seen — in mitosis — in Figure 78 ($a^{8.17}$, $a^{8.18}$), division is nearly completed, the spindles standing about vertically. By the continued overgowth of the anterior lip of the blastopore, the more dorsally situated of the daughter cells in the chorda fundament, e. g. $a^{8.17}$, are carried posterior to their sister cells, e. g. $a^{8.18}$ (cf. Fig. 79), and are finally entirely covered from sight by the nerve cells. They then form a plate of eight cells lying in the dorsal wall of the archenteron anterior to the blastopore.

The endoderm cells of this embryo (Plate XI. Fig. 78) have undergone no new divisions since the 128-cell stage (Figs. 73–77), though their nuclei have considerably increased in size, as is invariably the case during the resting period. More lateral sections than the one shown in Figure 78 exhibit spindles directed longitudinally in the mesenchyme cells $A^{8.12}$, $B^{8.12}$ (cf. Figs. 71 and 77), and show that division has been completed in $d^{7.12}$, $c^{7.12}$ (cf. Fig. 75).

Sections through two other embryos, a little more advanced still in development, show in the muscle cells $C^{7.6}$, $D^{7.6}$ (cf. Figs. 71 and 72) spindles directed forwards, inwards, and downwards, i. e. about toward the centre of the gastral cavity, a condition which is realized in the same cells at a corresponding stage in Clavelina. (See Van Beneden et Julin's ('86) Figs. 1 *a* and 1 *b*.) These facts will aid us in interpreting sections of later stages.

Figure 80 (Plate XI.) exhibits a dorsal view of a stage more advanced than any yet examined. The blastopore has greatly contracted (cf. Fig. 72) and now lies in the posterior half of the embryo. As gastrulation has progressed, there has taken place an ingrowth of cells round the margin of the blastopore into the inner layer of the embryo. We have already seen how by this process the anterior chorda cells attain a position in the dorsal wall of the archenteron; we may now observe that certain cells of the latero-posterior segments of the neuro-muscular ring are also involved in this ingrowth or invagination. At the stage shown in Figures 74–76 there was a double row of neuro-muscular cells on each side of the blastopore, now (see Fig. 80) the inner row is nearly covered from sight by the row of cells outside it.

Already at the stage shown in Figure 72 the most posterior neuro-muscular cells ($D^{7.6}$, $C^{7.6}$) had moved from their originally lateral position toward the median plane (cf. Fig. 71). There they were destined presently to meet each other, covering over the small mesenchyme cells $D^{7.5}$, $C^{7.5}$ (cf. Figs. 71 and 72 with Fig. 80); now (Fig. 80) they or their descendants lie at the posterior angle of the blastopore, and are in turn being covered over by the more laterally and anteriorly situated neuro-muscular cells.

The nerve cells anterior to the blastopore have increased considerably in number, perhaps through additions from the ectodermal group (cf. Figs. 72 and 80, also Figs. 78 and 79).

Three sections from a horizontal series through an embryo of about the stage shown in Figure 80 are represented in Plate XI. Figs. 81–83. The series consists of thirteen sections 6.67 μ thick, of which Figure 81 represents the third, Figure 82 the fifth, and Figure 83 the seventh. The sections are a little oblique, and consequently strike the right and left halves of the embryo at slightly different levels. The left side of Figure 82 shows best the history of the mesenchyme cells since the last stage examined in detail (Figs. 73–77). Lateral to the small gastral cavity we find the sister cells $B^{9.23}$, $B^{9.24}$, descendants of the common mother cell $B^{8.12}$ (Fig. 77). Evidence of the derivation of these two cells has been cited in the observation of a spindle longitudinally directed in the cell $B^{8.12}$ in two different embryos less advanced than this.

Lateral to $B^{9.23}$ and $B^{9.24}$ are the sister cells $B^{9.21}$, $B^{9.22}$, descendants of $B^{8.11}$ (Fig. 71; cf. $A^{8.11}$, Fig. 77). They stain more faintly than $B^{9.23}$ and $B^{9.24}$, a distinction which, it will be remembered, existed between the respective mother cells $B^{8.11}$ and $B^{8.12}$ (Fig. 77). Though a spindle has in no embryo been directly observed in $B^{8.11}$, evidence of the sistership of $B^{9.21}$ and $B^{9.22}$ (Fig. 82) exists in the still persistent interzonal filaments which stretch between their nuclei. This evidence is supported by the similarity of the cells in size and stainability. Posterior to the quartette of cells just discussed, the common descendants of $B^{7.6}$ (Plate X. Figs. 62, 67), are the two daughter cells derived from $c^{7.12}$ (Fig. 71), which was seen to be mitotic at an earlier stage (Fig. 75).

It was predicted from the position of the spindle in this cell that the division would be unequal, the more centrally and dorsally situated daughter cell being the smaller. This smaller cell is represented by $c^{8.24}$ (Fig. 82), but only the upper end of its large sister cell, $c^{8.23}$, appears in this section. In Figure 83 (Plate XI.) we see the deeper portion of $c^{8.23}$, which contains a nearly horizontal spindle.

Posterior to the pair of cells just described are the descendants of $C^{7.8}$ (Fig. 71), the next to the hindmost of the mesenchyme cells in the left half of the embryo. In Figure 74 they were in the eighth generation ($C^{8.16}$, $C^{8.15}$). One, the smaller, still remains in that generation ($C^{8.16}$, Fig. 82), but its larger, more deeply situated sister cell has passed into the ninth generation, and is now represented by $C^{9.30}$, $C^{9.29}$, Figure 83. The direct evidence of mitosis has not been observed for the division here assumed, but very strong indirect evidence for it exists in the fact that at the last cell division in the mesenchyme cells, $C^{7.8}$ divided earlier than $c^{7.12}$ (cf. Figs. 74 and 75). If the same order of division is followed in case of the daughter cells, division ought to occur earlier in $C^{8.15}$ than in $c^{8.23}$. But the latter cell is seen in Figure 83 to be in process of division; therefore it is reasonable to suppose that at the same stage the former cell has already divided.

The small posterior mesenchyme cells, $C^{7.5}$, $D^{7.5}$, lie one *behind* the the other deep down in the floor of the gastrula (Fig. 83), just posterior to the endoderm cells and overlaid by muscle cells, — for such the invaginated cells of the neuro-muscular ring become.

These muscle cells have been crowded inward and downward at the posterior margin of the blastopore in consequence of the rapid contraction of that opening.

In the most posterior pair of muscle cells, viz. $C^{7.6}$, $D^{7.6}$ (Fig. 71), mitosis was observed to occur, as already stated, at a stage earlier than this. The daughter cells arising from that division are readily recognized in $C^{8.11}$, $C^{8.12}$, and $D^{8.11}$, $D^{8.12}$ (Fig. 83). The nuclei of $C^{8.11}$ and $D^{8.11}$ lie in the section intermediate between those represented in Figures [illegible]

I am not able to declare with certainty the [illegible] of the other muscle cells in this series of sections, so I shall not attempt to [illegible] them out one by one. As a group, however, they are clearly distinguished from the ectoderm cells on the one hand, and from the mesenchyme cells on the other, by their large nuclei, their considerable size, and the peculiar stainability of their protoplasm. They resemble very closely in stainability the nerve cells lying anterior to the blastopore, but they are much larger than the nerve cells. They now lie lateral and posterior to the blastopore. (See Figs. 81–83; cf. Figs. 79 and 80.)

The backward growth of the anterior lip of the blastopore has carried the crescent-shaped anterior chorda fundament (Fig. 71) from its original position to about the middle of the embryo's dorsal surface (Fig. 81). It was seen in Figure 71 to consist of eight cells, which have now increased (Figure 78) to sixteen, and lie crowded together in two rows, one superposed above and overhanging the other (cf. Figs. 79 and 81.

In Figures 62 (Plate X.) and 71 (Plate XI.) we saw that the two posterior chorda cells, viz. $c^{7.11}$, $d^{7.11}$, were separated from the anterior chorda cells by the mesenchyme cells, $B^{7.6}$, $A^{7.6}$, or their descendants. In Figure 82 we see that the descendants of $B^{7.6}$, $A^{7.6}$ (viz. $B^{9.21}$, $B^{9.22}$, $B^{9.23}$, $B^{9.24}$, and the corresponding cells in quadrant A) during the process of invagination have been pushed down to the level of the other mesenchyme cells, allowing the anterior chorda cells to come into contact with the isolated posterior chorda cells (Fig. 81, $c^{8.21}$, $c^{8.22}$) above them. The posterior chorda cells were seen to be in the seventh generation in Figure 71 ($c^{7.11}$, $d^{7.11}$). At the stage represented in Figure 81, there is good reason to believe that they have divided and passed into the eighth generation, since every other cell of the dorsal hemisphere is known to have done so previous to that stage; they are therefore represented by the cells $c^{8.21}$, $c^{8.22}$, $d^{8.21}$, and $d^{8.22}$, the last named cell being hidden from view in Figure 81 by the overlying muscle cell.

The endoderm cells still remain in the eighth generation, and number twenty. Their arrangement is made clear by an examination of Figures 81–83, in comparison with Figure 79, which represents a section near and parallel to the median plane of a slightly earlier stage. Fourteen of the twenty endoderm cells abut on the median plane, and six are placed laterally toward the anterior end of the embryo. The median double row of cells consists of $b^{7.11}$ (Fig. 81), $b^{7.12}$ (Fig. 82), $b^{7.15}$, $b^{7.16}$, $c^{7.16}$, $c^{7.14}$, $c^{7.13}$ (Fig. 83), and the corresponding cells in the right half of the embryo. The nuclei do not appear in the centrally and posteriorly situated endoderm cells of Figure 83 because they lie in later (deeper) sections of the series, not figured (cf. Fig. 79). Only the narrow upper ends of the cells in question appear in Figure 83, which therefore gives no adequate idea of their size, but a correct idea of this may be had by an examination of Figure 79. The laterally situated endoderm cells are $c^{7.9}$ (Fig. 82), $c^{7.10}$, $c^{7.15}$ (Fig. 83), and the corresponding cells in the right half of the embryo.

From a series of cross sections through an embryo in about the same stage as is represented in Figure 80, four sections have been selected to make more clear the relations of the fundaments of the various organs. (See Plate XII. Figs. 84–87.) Figure 84 represents a section just behind the blastopore (compare with it Plate XI. Fig. 73); in it the ectoderm is seen to have slightly overgrown from behind the most posterior muscle cells. (Compare Fig. 79, Plate XII.) Only one of the pair of small posterior mesenchyme cells ($D^{7.5}$, $C^{7.5}$) appears in the section; the other lies in the section just posterior to this.

Mitosis is again setting in among the endoderm cells, as is shown by the spindle in $d^{7.13}$; the next section anterior to this likewise shows spindles in the endoderm cells that are cut, $c^{7.14}$ and $d^{7.14}$. The spindles in each case ($^{7.13}$ and $^{7.14}$) are directed longitudinally, and in such a manner that the eight resulting cells will all lie in a single slightly concave layer. The consequence of these divisions will be a considerable elongation of the double row of endoderm cells at the posterior end of the embryo.

It is worthy of note, though not shown in this series of sections, that at this division, as in the preceding and in subsequent ones, the spindles of the endoderm cells do not lie in the longest axis of the cells, which is the vertical.

Figure 85 shows a section through the still open blastopore at its posterior margin. A comparison of this figure with Figures 72, 74, and 75 (Plate XI.) shows that the ectoderm has grown rapidly in superficial extent through cell multiplication, and shoved the neuro-muscular cells $C^{9.13}$, $D^{9.13}$, inward to a position overlying their sister cells, $C^{9.14}$, $D^{9.14}$. The small mesenchyme cell, $C^{8.16}$ (cf. Plate XI. Fig. 74, $D^{8.16}$), is in process of division, following the lead of its large sister cell, $C^{8.15}$ (cf. Figs. 74, $D^{8.15}$, and 84, $D^{9.29}$, $D^{9.30}$). The mate of $C^{8.16}$, viz. $D^{8.16}$, has already divided. One of its daughter cells is seen in this section ($D^{9.32}$, Fig. 85), the other lies in the next posterior section. The large mesenchyme cells, $c^{8.23}$, $d^{8.23}$ (Fig. 85), are in mitosis (cf. Plate XI. Fig. 83).

The section represented in Figure 86 encounters the blastopore farther forward than the one last described (Fig. 85), in its broader portion (cf. Plate XI. Figs. 72, 76, and 77). Here, too, the muscle cells have been crowded inward and partially invaginated; $C^{9.15}$ and $D^{9.15}$ overlie their sister cells, $C^{9.16}$ and $D^{9.16}$, respectively. Of the posterior chorda cells only $c^{8.22}$ and $d^{8.22}$ appear in this section. Their more laterally placed sister cells, $c^{8.21}$ and $d^{8.21}$, lie in the next posterior section (not figured), and at a slightly higher level (cf. Fig. 81, Plate XI.).

Figure 87 (Plate XII.) represents the first section anterior to the blastopore (cf. Plate XI. Fig. 80). On its depressed dorsal surface are seen six cells of the anterior chorda fundament, which is being rapidly covered over from the sides and anterior end by the ectoderm. Extending deep down on either side of the chorda appears a deeply stained cell (stippled in the drawing) with large nucleus. These two are the most posterior cells of the medullary plate, which now lies at the dorsal surface of the embryo anterior to the blastopore, having been formed chiefly by the anterior segment of the neuro-muscular ring (cf. Plate XI. Fig. 80).

The mesenchyme cells, $A^{9.24}$, $B^{9.24}$ (Fig. 87), are seen to lie on each side of the gastrula cavity (cf. Plate XI. Fig. 82); lateral to them lie the relatively small and faintly stained mesenchyme cells, $A^{9.22}$, $B^{9.22}$. The respective sister cells of those just mentioned, viz. $A^{9.23}$, $B^{9.23}$, $A^{9.21}$, and $B^{9.21}$ (cf. Plate XI. Fig. 82), lie in the next two posterior sections (not figured).

The stage next to be discussed differs in external appearance from that shown in Figure 80 chiefly, first, in the further contraction of its blastopore to a small aperture in the dorsal surface somewhat posterior to its centre; secondly, in a slight elongation of the embryo and narrowing of its posterior end, foreshadowing the formation of the tail; and thirdly, in a slight depression of the medullary plate to form a neural or medullary groove (cf. Fig. 98).

From a series of transverse sections through an embryo in this stage, five are represented in Plate XII. Figs. 88–92. Figure 88 (Plate XII.) shows a section posterior to the blastopore (cf. Fig. 98). It passes through the region of the small posterior mesenchyme cells, $C^{7.5}$, $D^{7.5}$. Lateral or dorsal to them are seen four pairs of muscle cells containing large nuclei. The finely granular cytoplasm of these muscle cells takes a deep grayish blue stain in hæmatoxylin. Bounding the whole section is the uninterrupted ectoderm.

The next anterior section, which has nearly twice the area of this, is likewise completely surrounded by ectoderm.

The second section anterior to the one shown in Figure 88 is represented in Figure 89 (Plate XII.). Two endoderm cells, the most posterior ones, appear in it. The small size of their nuclei indicates that they belong to a later generation than the endoderm cells seen in Figure 84. Unquestionably they are in the eighth generation. To right and left of them appear two muscle cells, probably descendants of $C^{7.6}$, $D^{7.6}$, Plate XI. Fig. 73 (cf. Plate XI. Fig. 83, $C^{8.11}$, $C^{8.12}$, $D^{8.11}$, and $D^{8.12}$). Lateral to the muscle cells mentioned are seen in Figure 89

(Plate XII.) mesenchyme cells, three on each side. Two of them are undoubtedly descendants of the mesenchyme cells $C^{8.15}$, $D^{8.15}$, shown in Plate XI. Figs. 73 and 74, and represented in Plate XI. Fig. 83, and Plate XII. Fig. 84, by the cells $C^{9.29}$, $C^{9.30}$, $D^{9.29}$, and $D^{9.30}$. Their nuclear condition shows that they have arisen from a recent division. Dorsal to the groups of cells already mentioned are seen in Figure 89 muscle cells extending up in a solid mass to the dorsal surface of the embryo. In the mid-dorsal surface of the section is a pair of cells, probably nerve cells, between which at an earlier stage lay the open blastopore. The periphery of the section is elsewhere bounded by ectoderm.

Figure 90 (Plate XII.) represents the second section anterior to that shown in Figure 89. It passes through the widest portion of the blastopore. The only other section of the series which passes through the blastopore is the next preceding one, in which the blastoporic opening is extremely narrow, in fact, scarcely more than a slit. Figure 91 shows the first section anterior to the blastopore. In it we see a plate of seven cells (*cd.*) belonging to the anterior chorda fundament and forming the roof of the archenteron (cf. Fig. 81). In Figure 90 we find the posterior chorda cells (*cd.*) lateral to the blastopore (cf. Plate XI. Fig. 81, $c^{8.22}$ and $c^{8.21}$). Ventral to the chorda cells in Figures 90 and 91 are the mesenchyme cells descended from $c^{8.23}$, $c^{8.24}$, $d^{8.23}$, and $d^{8.24}$ (cf. Plate XI. Figs. 82 and 83).

Dorsal to the chorda cells in Figure 91 are four cells unquestionably nervous, the two lateral ones being large and in mitosis, the other two small, evidently produced by recent divisions. In the next anterior section (not figured) the two lateral mitotic nerve cells again appear; completely filling the space between them are four small nerve cells similar to the two seen in Figure 91. A medullary groove is thus clearly formed anterior to the blastopore, and the four cells dorsal to the chorda fundament in Figure 91 evidently are only lateral backward prolongations of the medullary plate. The two large cells at the margins of the blastopore in Figure 90 are probably $C^{9.15}$ and $D^{9.15}$ (cf. Fig. 86); their deeper lying sister cells $C^{9.16}$ and $D^{9.16}$ have been carried into the more posterior sections by the crowding backward of the chorda cells and the elongation of the embryo.

Figure 92 (Plate XII.) represents the third section anterior to the one shown in Figure 91. The medullary plate and chorda are here represented each by four cells. The mesenchyme cells visible on each side of the archenteron are $A^{9.23}$, $B^{9.23}$, $A^{9.21}$, and $B^{9.21}$ (cf. Plate XI. Fig. 82). The cells $A^{9.24}$, $B^{9.24}$, $A^{9.22}$, and $B^{9.22}$ (cf. Fig. 82) lie in the next two anterior sections. Of the eight cells mentioned, $A^{9.23}$, $A^{9.24}$, $B^{9.23}$, and $B^{9.24}$ (cf. Fig. 82) are all in mitosis, but the four more laterally situated and smaller ones are still quiescent.

Considering as a whole the mesenchyme of this embryo, we see that it consists of two lateral bands which have elongated with the elongation of the embryo. They now extend through ten different sections from near the anterior end of the embryo to a region posterior to the blastopore (Fig. 89). The muscle cells, on the other hand, are gathered into a pretty compact mass at the sides of and posterior to the blastopore (Figs. 88–90).

In the subsequent stages of development the portion of the embryo lateral and posterior to the blastopore will be rapidly drawn out to form the tail of the larva, while the portion anterior to the blastopore will form the trunk. This will not come about, however, without a considerable shifting of cells from one portion into the other, for the chorda cells, which now lie anterior or lateral to the blastopore, must in large part pass into the tail, while the mesenchyme cells, which are more ventrally located, and some of which now extend behind the blastopore, will all pass forward into the trunk region.

An examination of Figure 98 (Plate XII.) may help to give a clearer idea of the stage just described. This figure shows a section made nearly parallel to the sagittal plane, but a little to one side of it, through an embryo slightly older than the one last under discussion (Figs. 88–92). The anterior chorda fundament, it is seen, has been carried back beyond the middle of the embryo's dorsal surface. The muscle cells have been forced backward and downward into a nearly vertical position behind the blastopore, and are nearly covered over with ectoderm (cf. Fig. 93).

Numerous cell divisions have recently occurred in the ectoderm, and the number of endoderm cells has also plainly increased. A very marked elongation of the embryo has attended these divisions. Several cells in the medullary plate are also dividing. On account of the slight obliquity of the plane of sectioning, the small posterior cells $C^{7.5}$, $D^{7.5}$ (*ms'chy.*), do not actually appear in this section as represented, but have been projected there from the adjacent section. In that section the endoderm extends back in a double row of cells into contact with $C^{7.5}$, $D^{7.5}$, as at the stages shown in Plate XI. Figs. 78 and 79.

In Figures 93–97 (Plate XII.) are represented five cross sections through an embryo in about the same stage as is shown in Figure 98. The approximate position of the sections in the embryo is indicated on

Figure 98 by the five vertical lines 93–97. Figure 93 shows a section posterior to the blastopore. It passes through one (*ms'chy.*) of the small posterior mesenchyme cells, $D^{7.5}$, $C^{7.6}$ (cf. Fig. 88), the other lying in the next section posterior to this. The interior of the section is filled with a solid mass of muscle cells, or more properly nerve cells and muscle cells; for it is highly probable that the four most dorsally situated of these cells, which form a group not quite covered in by the ectoderm, are to become part of the nerve cord of the tail (cf. Plate XIII. Figs. 99–101, *n.*). However, they are not distinguishable in histological characters from the more laterally and ventrally situated cells of the section. Cell division has recently occurred in the ectoderm, which plainly is soon to cover in completely the nerve cells in this region of the embryo. The muscle cells have evidently been reduced in size by division since the stage shown in Figures 88 and 89.

The second section anterior to this is shown in Figure 94. It is the only section of the series which passes through the blastopore, now reduced almost to a slit.

The blastopore is bordered on each side dorsally by a large nerve (?) cell, *n.* (cf. Fig. 90). Ventral to the nerve cells lie the posterior chorda cells, *cd.*, lateral and still ventral to which are muscle cells. The most posterior pair of endoderm cells lies underneath the open blastopore, and a single small mesenchyme cell lies deep down in each half of the section.

The second section anterior to the blastopore is shown in Figure 95; the second section anterior to that, in Figure 96; and one situated still two sections farther forward, in Figure 97.

In Figure 96 the medullary plate is not at all depressed at its centre; it consists of four large cells closely packed together and columnar in form. In Figure 97 the medullary plate is not even flattened, but conforms to the evenly rounded contour of the embryo in that region. It consists of six cells sharply distinguished from the cells of the ectoderm in stainability, though the size of the more lateral ones is not materially different from that of the ectoderm cells. The chorda plate has diminished to a breadth of only three cells in Figure 96, and is entirely wanting in Figure 97, where endoderm cells occupy the space dorsal to the archenteron underneath the medullary plate. The mesenchyme bands cover considerable area in Figure 96, but are reduced to a single cell on each side of the body in Figure 97, from which it is seen that in this region the interior is nearly filled with a solid mass of endoderm. The section represented by Figure 97 lies well toward the anterior end of the embryo, as is indicated by the rapidly diminishing size of the sections.

To summarize our observations on the series of sections just examined (Figs. 93–97): —

(1) The fundament of the *nervous system* consists of a medullary plate extending from near the anterior end of the embryo to the blastopore, and continued backward by cells lying on each side of the blastopore and along the line where the lips of the blastopore have fused. The transformation of the medullary plate into a medullary groove proceeds from the blastopore forward.

(2) The *chorda* fundament consists of a plate of cells immediately underneath the medullary plate, but extending neither so far forward nor so far backward in the embryo. A part of it lies on each side of the blastopore, but the larger part is anterior to the blastopore.

(3) The *mesenchyme* extends in two lateral bands from the region of the blastopore forward through about two thirds of the extent of the embryo anterior to the blastopore.

(4) The *muscle cells* lie principally posterior to the blastopore in a pretty compact mass. They extend no farther forward than the first section anterior to the blastopore.

(5) The *endoderm* consists of a double row of large cells ventrally situated extending from the first section behind the blastopore through the next five anterior sections; it then broadens out and occupies nearly the whole inner layer of the embryo, both dorsally and ventrally, anterior to the chorda fundament.

C. SUMMARY ON GASTRULATION.

1. In the gastrulation of Ciona two processes can be distinguished: (*a*) a progressive invagination of the cells on the dorsal surface of the embryo, beginning at its centre; (*b*) a concomitant overgrowth of cells from the ventral side of the embryo, caused by more rapid cell division in that region. The overgrowth is greater at the anterior than at the posterior end of the embryo, because cell division proceeds more rapidly at the anterior end.

2. Early in the process of gastrulation one can recognize a ring of cells encircling the blastopore peculiar in their stainability, forming the common fundament of the nervous system and the longitudinal musculature of the larva.[1] Anterior to the blastopore the ring broadens out

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[1] The existence of this peculiar ring of cells was first pointed out by Van Beneden et Julin ('86) in the case of Clavelina; but these authors made the mistake of regarding it as exclusively nervous.

to form the medullary plate. Those cells of this *neuro-muscular* ring which lie on each side of and posterior to the blastopore are for the most part invaginated, and form the entire longitudinal musculature of the tail. Some of them, however, form the most posterior portion of the nerve cord.

3. Lying just within the margin of the blastopore, and encircled by the neuro-muscular ring, is another ring of cells, interrupted at the posterior end of the embryo only. Its anterior portion gives rise to the greater part of the chorda; its remaining (lateral) portions produce the mesenchyme or trunk mesoderm, besides contributing to the chorda a single cell at each lateral margin of the blastopore. The descendants of these two chorda cells meet in the median plane at the closure of the blastopore. They form the most posterior portion of the chorda.

We may regard the chorda-mesenchyme ring as being completed morphologically by the two small sub-chordal mesoderm cells $C^{7.5}$, $D^{7.5}$, which have been wedged in between the most posterior cells of the neuro-muscular ring. Like the other cells of the chorda-mesenchyme ring, they lie in contact with the endoderm cells on one side, and with cells of the neuro-muscular ring on the other. Ultimately they probably form mesenchyme in the tail region. Possibly by a cœnogenetic reduction in size to their present minute dimensions, a gap has been left on each side of the embryo between them and the lateral portions of the chorda-mesenchyme ring. This change may have attended a cœnogenetic lengthening of the posterior end of the organism to subserve locomotion. There is evidence from other sources that the trunk of Ascidians formerly extended farther back into what is now the tail region of the larva. At that time the mesenchyme also probably extended farther back, and the chorda-mesenchyme fundament was in ontogeny, as we suppose it to have been in phylogeny, an uninterrupted ring.

4. The blastopore, at first widely open, closes more rapidly from the anterior margin and from the sides than from behind. Consequently it comes to lie in the posterior portion of the dorsal surface of the embryo, and is triangular in form. The right and left sides of the triangular blastopore, however, fuse from behind forward, beginning in the region of the pair of small, flattened mesoderm cells, $C^{7.5}$, $D^{7.5}$. Along the line of union of the lateral lips of the blastopore lies superficially on each side of the median plane a row of nerve cells. These are subsequently covered in by ectoderm from the sides and from behind, and form the posterior portion of the nerve cord. Underneath them, and at first not distinguishable from them in histological characters, are other cells, likewise derived from

the posterior portion of the neuro-muscular ring; these are destined to form the longitudinal musculature of the tail. The medullary plate, which produces the entire nervous system of the *trunk* region, lies wholly anterior to the region of "concrescence" of the lips of the blastopore.

5. The posterior margin of the blastopore does not grow forward over the blastopore covering in the medullary canal as described by Van Beneden et Julin ('86) in the case of Clavelina.[1]

6. I heartily concur in Samassa's ('94) conclusion that there is no rotation of axes during the gastrulation of Ciona, such as Korschelt u. Heider ('93), on theoretical grounds, conjectured might occur in Ascidians. Their hypothesis is, so far as I know, entirely unsupported by observation.

4. Formation of the Larva.

The further changes which the embryo undergoes in its transformation into the larval tadpole will be understood from an examination of Figures 99–105 (Plate XIII.), which represent seven sections through an embryo with completely closed blastopore. Figure 99 shows the third section (in passing from behind forward) of the series; it contains about half a dozen muscle cells and four nerve cells, surrounded by an epithelial layer of ectoderm. The first section of the series shows merely the ectoderm cut tangentially; the second contains six muscle cells surrounded by the ectoderm, but no nerve cells or chorda. The four nerve cells in Figure 99 show precisely the same arrangement as is found later in a cross section of the tail of the larva. (See the four cells at the right of *cd.* in the right portion of Fig. 106.)

In Figure 100 (Plate XIII.) the number of nerve cells (seven) is seen to be increased, and the chorda makes its appearance as a group of seven cells ventral to the nerve cells.

In Figure 101 (Plate XIII.) the nervous and chorda fundaments appear about as in the section shown in Figure 100, but underneath the chorda is seen a group of four small mesoderm cells, the descendants of $D^{7.5}$, $C^{7.5}$ (Plate XII. Fig. 88), which have at last divided. Just anterior to them in the embryo (Figs. 102 and 103) extends the double row of caudal endoderm cells. As I have already suggested (page 262), the subchordal mesoderm cells (Fig. 101) probably have the same fate as the caudal endoderm cells, i. e. are resolved into mesenchyme at a later stage.

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[1] The authors mentioned were doubtless led into this mistaken interpretation by identifying as nerve cells the *muscle* cells which lie behind the blastopore at the time of its closure. (See their Figs. 1 *a*, 1 *c*, 2 *c*, 3 *a*, Pl. VII. These figures are reproduced in Korschelt u. Heider's ('93, Figs. 741 A, 741 B, 742 B, and 745 B, respectively.)

Figure 102 (Plate XIII.) represents a section through what probably was the region of the blastopore. In it is seen the most posterior pair of endoderm cells (cf. Plate XII. Fig. 94). Since the stage last examined (Plate XII. Figs. 93–97) the chorda cells have closed together into a single plate in this region, and the chorda fundament has grown farther back in the embryo. The nerve cells which lay at each side of the blastopore (Plate XII. Fig. 94) have also met in the median plane to form a single plate, which is now closing into a canal. A real canal is never formed posterior to the blastopore, though the nerve cells in that region potentially form one.

Figure 103 (Plate XIII.) represents the second section anterior to the one shown in Figure 102; Figure 104, the second anterior to that; and Figure 105, the fourth anterior to that. It will be seen that the muscle cells which in the series last examined (Figs. 93–97) were aggregated chiefly behind the blastopore, have now extended themselves not only posterior, but also anterior, to the blastopore. They extend as far forward as the next section in front of the one represented by Figure 103, i. e. through three sections anterior to the blastopore. They have pushed before them the mesenchyme, which in this series first appears in the section shown in Figure 103. The chorda fundament has meanwhile moved toward the posterior end of the embryo. It now extends two sections behind the blastopore and overlies the small posterior mesenchyme cells (Fig. 101, cf. Plate XII. Fig. 93). Accompanying the changes just mentioned, has come a diminution of the diameter of the embryo at its posterior end, which is already elongating to form the tail region.

The mesenchyme extends forward of the section shown in Figure 103 through six sections. The medullary plate extends forward two or three sections farther still. The endoderm consists of a double row of cells extending forward underneath the chorda as far as the section seen in Figure 104, in which four endoderm cells are found; the arrangement there shown has been derived from that shown in Plate XII. Fig. 96, and still earlier in Plate XII. Fig. 91, by the meeting in the median plane underneath the chorda of the more laterally placed endoderm cells. Later, these four cells, or their descendants, will move apart so as to enclose between them the lumen of the posterior portion of the digestive tract. Anterior to the section shown in Figure 104 the endoderm rapidly increases in amount, while the chorda and mesenchyme diminish.

In the region shown in Figure 105, it fills the entire interior of the section.

In Figure 106 (Plate XIII.) is shown a section through an embryo in which the tail is already recognizable as a distinct portion of the embryo, though it has not yet reached anything like its maximum length. It is curved ventrad under the trunk, so that the section passes transversely through both trunk and tail. The section passes through the trunk in the brain region, but intersects only one of the mesenchyme bands, the other one not extending so far forward in the embryo. The endoderm cells are seen to have arranged themselves round a potential lumen in the form of an epithelium. However, they still lie two deep in places. Their shape is clearly becoming columnar.

In the tail region appears the chorda, now transformed into a single row of flattened, disk-shaped cells, rapidly becoming vacuolated. They form an axial rod extending through the entire tail region and the posterior portion of the trunk. Dorsal (right in the Figure) to the chorda lies the nerve cord of the tail, composed in cross section of about four small cells.

Ventral to the chorda is the sub-chordal endoderm strand consisting of a double row of cells (*en'drm.*). On each side of the chorda are seen in the section about three muscle cells.

Summary on Formation of the Larva.

1. The nerve cord in the limited region of concrescence of the lips of the blastopore is covered over by the ectoderm first at its posterior end and then successively in its more anterior regions, following the course of concrescence. The nerve cells in that portion of the embryo never form a *real* canal, but only a *potential* one. They are arranged in a solid strand, which usually shows in cross section four cells placed round a common centre, the potential canal.

The medullary plate arises wholly anterior to the blastopore. At the time when the blastopore is about to close, the medullary plate has come to extend over a great part of the length of the embryo, and has sunk down in the form of a shallow grove deepest at its posterior end, the anterior margin of the blastopore. When the blastopore closes, it begins to form a canal. This process, like the fusion of the lateral margins of the blastopore, advances from behind forward.

2. Beginning shortly before the closure of the blastopore, a rapid elongation of the embryo takes place, accompanied by a considerable change in its form and a rearrangement of the cells composing some of

its organs. The posterior end of the embryo, which toward the completion of gastrulation was broader than the anterior end, becomes narrower and narrower, and ultimately forms the tail, which is curved ventrad around the trunk of the embryo within the egg membranes.

Before the closure of the blastopore the chorda is a plate of cells lying in the dorsal wall of the archenteron, anterior and lateral to the blastopore. The portions lateral to the blastopore meet in the median plane when the blastopore closes. The chorda fundament then elongates, owing to a shoving together of its cells from each side, "like a pack of cards in shuffling" (Van Beneden et Julin), until they form, instead of a plate, a single median row of disk-shaped cells arranged one behind another like a row of coins and reaching backward underneath the nerve cord to the extreme posterior end of the embryo. Anteriorly it terminates not far from the middle of the trunk region.

The muscle cells, which originally lay on each side of and behind the blastopore, extend themselves a single cell deep in two bands, one on each side of the chorda throughout its entire length.

The mesenchyme cells originally formed the lateral portions of the chorda-mesenchyme ring. As the blastopore gradually closed, they were thrust down to a deeper level than the muscle cells, and forward. Ultimately they come to lie wholly in the trunk region, chiefly in its posterior portion, in two pretty compact lateral masses of small deeply stained cells, two or more layers deep. At a still later period, these lateral masses are resolved into migratory cells, i. e. blood corpuscles, mantle cells, etc.

Before the closure of the blastopore the endoderm forms the entire lining of the archenteron in its most anterior portion, where its lumen is almost obliterated. Farther back the chorda forms the dorsal wall of the archenteron, the mesenchyme cells forming its sides, the floor only being occupied by the endoderm cells. In the region where the blastopore closes, the endoderm cells occur only as a double row ventrally situated along the median line.

This double row is extended back in the larva underneath the chorda throughout almost the entire length of the tail, forming a "subchordal endoderm strand," which is ultimately resolved into wandering cells, or perhaps utilized as food material by the mesenchyme cells of the trunk region. At the posterior end of this caudal endoderm strand lie the small mesoderm cells which Van Beneden et Julin mistakenly included in the nervous fundament. These cells are to be regarded as the most posterior constituents of the original chorda-mesenchyme ring.

Like the cells of the endoderm strand just anterior to them, they probably become wandering cells.

In the posterior portion of the trunk region, where before the closure of the blastopore the endoderm strand broadens out into a plate of four or more cells, the more laterally placed endoderm cells move dorsad at the closure of the blastopore, and meet in the median plane underneath the chorda. In this way the endoderm of the trunk region is converted into a closed vesicle, pear-shaped and broadest in its anterior portions; at its posterior end it is overlaid by the chorda and flanked on each side by the mesenchyme.

VII. DISCUSSION OF SOME THEORETICAL QUESTIONS.

The facts presented in the foregoing pages have a certain bearing on several questions of general interest. Of these I shall make brief reference to, — 1. The origin of the germ layers of Chordates; 2. The Cœlom theory; and 3. The ancestry of Chordates.

A. Origin of the Germ Layers of Chordates.

According to the generally accepted doctrine of Haeckel, all the higher metazoa are ultimately derived from a simple cup-shaped or sac-like ancestor composed of two cell layers, an inner and an outer, continuous with each other at the margin of the cup or sac. The two cell layers are called the primary germ layers. The outer layer is known as the primary ectoderm; the inner, as the primary endoderm. Among the Chordates, this supposed ancestral condition is most nearly realized in ontogeny in the case of Amphioxus. The homologues of its inner and outer germ layers are traced by embryologists through all the groups of the chordate phylum. A third or middle layer, derived from one or both of the others, makes its appearance between the two primary germ layers in all the higher Metazoa. Whether this middle layer, or mesoderm, is homologous throughout the different groups of Metazoa is one of the most difficult and disputed questions in the whole realm of comparative embryology. Into this question I do not propose to go in this paper; I shall confine my attention to the mesoderm of Chordates.

It is commonly believed that the mesoderm of Chordates is derived entirely from the inner germ layer, which is accordingly often referred to as mes-endoderm. With this view, however, my observations on Ciona force me to take issue. In an earlier part of this paper it has been shown that during the process of gastrulation in Ciona there is a progressive ingrowth of cells around the blastopore from a position in the outer to a position in the inner layer of the gastrula. Whether, therefore, we shall include a particular cell in the primary ectoderm or primary endoderm depends on whether we consider the embryo at an earlier or a later period in the process of gastrulation. Lwoff ('94) has recently stated, and it seems to me on excellent evidence, that in Amphioxus and all Vertebrates there occurs in the formation of the germ layers an ingrowth of cells from the outer to the inner layer of the embryo, very similar to that which I have observed in Ciona. He accordingly distinguishes what we may call a *primary invagination* of the cells destined to form chiefly the alimentary tract from a *secondary invagination* involving the cells destined to form the chorda and a portion of the mesoderm, viz. the musculature. The matter seems to me of sufficient importance to warrant the quotation of the author's own words. Speaking of Amphioxus he says: —

"Ich bin also zum Schluss gekommen, dass *die dorsale Wand der Gastrulahöhle, die ich als dorsale Platte bezeichnen will, von den Ektodermzellen gebildet ist.* Dieses Ergebniss ist von sehr grosser Bedeutung. Wie die weiteren Entwickelungsstadien lehren, stellt diese dorsale Wand die Anlage der Chorda und des Mesoderms dar, indem aus der mittleren Zellenpartie derselben die Chorda, aus zwei seitlichen Theilen das der Chorda anliegende Mesoderm entsteht, aus welchem, wie bekannt, die Muskelelemente entstehen. Dies zeigt, das *die Chorda und das anliegende Mesoderm aus einer ektoblastogenen Anlage entstehen,* die ursprünglich als eine zusammenhängende Platte (dorsale Platte) erscheint. Was die eigentlichen Entodermzellen betrifft, welche die übrige Wandung der Höhle bilden, so will ich hier in Kürze vorbemerken, dass sie jederseits einige an die dorsale Platte angrenzende Zellen als ihren Beitrag zur Bildung des Mesoderms abgeben; die Ränder des übriggebliebenen Entoderms wachsen unter den seitlichen Mesodermanlagen nach der Mittellinie zu, vereinigen sich unter der Chorda und bilden auf solche Weise den Darm.

"*Das Hauptergebniss* dieser Untersuchungen ist, *dass die Einstülpung bei Amphioxus keineswegs als eine einfache Gastrulation zu betrachten ist,* wie es bisher angenommen. *Es sind vielmehr hier zwei verschiedene Processe zu unterscheiden: erstens die Einstülpung der Entodermzellen, aus denen der Darm entsteht; zweitens die Einstülpung der Ektodermzellen vom dorsalen Umschlagsrande aus, welche die ektoblastogene Anlage der Chorda* *und des Mesoderms bildet. Die Einstülpung der Entodermzellen ist als Gastrulation zu betrachten. Es ist ein palingenetischer Process,* den die Chordaten von ihren Vorfahren ererbt zu haben scheinen, wo dieser Process gleichmässig und radial symmetrisch vor sich ging, wie es sich bei einigen wirbellosen Thieren beobachten lässt. *Die Einstülpung der Ektodermzellen ist dagegen als ein coenogenetischer Process zu betrachten, der mit der Bildung des Darmes nichts zu thun hat und durch den die Bildung der ektoblastogenen Anlage der Chorda und des Mesoderms eingeleitet wird.* Wie in den folgenden Abschnitten gezeigt werden soll, lassen sich diese zwei Processe — die Bildung des Darmes und die Bildung der ektoblastogenen Anlage der Chorda und des Mesoderms — auch in der Entwickelung aller Wirbelthiere von einander unterscheiden." (Separate, pp. 11, 12.)

Whether Lwoff is right in including the chorda and the greater portion of the mesoderm of Amphioxus and the Vertebrates in the secondarily invaginated part of the inner germ layer, I do not attempt to say. That question must be decided by an examination of the forms on which he made his observations. However, in Ciona, at least, the cells which are destined to form chorda and mesenchyme (chorda-mesenchyme ring) must be regarded as taking part in a *primary invagination* along with the definitive endoderm. But plainly a very important part of the mesoderm, viz. the cells which form the longitudinal musculature, is carried into the inner layer by a *secondary* invagination. The secondarily invaginated cells are derived from the posterior segments of the neuromuscular ring. At the beginning of gastrulation they clearly lie in the outer layer of the gastrula, but at the conclusion of gastrulation they lie within the margin of the blastopore.

Accordingly, I regard the definitive endoderm fundament and the encircling chorda-mesenchyme ring as constituting the *primary endoderm* in Ciona. In the *primary ectoderm*, I would include the neuro-muscular ring and the "ectodermal group" of cells, both of which lie entirely in the outer layer of the gastrula when the closure of the blastopore begins. If this view is correct, the mesoderm or middle germ layer of Ascidians must be regarded as derived in part from the primary endoderm and in part from the primary ectoderm. Lwoff reached a similar conclusion concerning the origin of the mesoderm in Amphioxus and the Vertebrates. In Ciona that part of the mesoderm which is derived from the outer germ layer produces the longitudinal musculature of the larva. It forms the whole of this tissue, and nothing else. Similarly in Amphioxus and the Vertebrates, Lwoff concluded that the ectodermal mesoderm formed the longitudinal musculature. It would seem, therefore, that the *muscle plate* of the mesodermal somite of Amphioxus is homologous with the muscle cells of the Ciona tadpole. Both in Amphioxus and in Ciona the muscle fundament arises from cells lying lateral to the chorda and derived from the primary ectoderm. In Amphioxus the musculature, like the chorda with which it is intimately associated, becomes (cœnogenetically?) extended far forward to the anterior end of the trunk region; whereas in Ciona neither musculature nor chorda extends farther forward than about the middle of the trunk region.

The mesoderm lateral to the muscle plates of Amphioxus seems to be the homologue of the mesenchyme of Ciona. Both are derived from the *endodermal* portion of the mesoderm. (Cf. the quotation from Lwoff, pages 268, 269.)

My conclusions differ from those of Lwoff chiefly regarding the origin of the chorda. He considers this organ to be derived from the primary ectoderm in Amphioxus and the Vertebrates, whereas I regard it as formed in Ciona exclusively by the primary endoderm. I think that Lwoff has been led to include the chorda cells in the primary ectoderm chiefly because they are in Amphioxus (as in Ciona) smaller and clearer than the less rapidly cleaving endoderm cells. These criteria I regard as insufficient. Only a study of the cell lineage can give in any case a *positive* answer to the question whether the chorda cells at the beginning of gastrulation lie in the outer or the inner layer of the embryo.

That a distinction is rightly made in the case of Ascidians between the two kinds of mesoderm which I have recognized, viz. musculature and mesenchyme, is unanimously agreed to by embryologists; *but the fact has been heretofore overlooked that these two kinds of mesoderm are derived from different fundaments early distinguishable both histologically and topographically, and that these fundaments should be regarded as derived from different primary germ layers.*

A minor point of theoretical importance is whether or not the chorda shall be regarded as a *mesodermal* organ. Lwoff does not so consider it, though he recognizes two facts which, it seems to me, would naturally lead one to that conclusion: the first, that in Amphioxus and the lower groups of Vertebrates the chorda is derived from a common fundament with what is universally regarded as mesoderm; the second, that the chorda, like the undoubted mesoderm, comes to occupy a position between the inner and outer layers of the embryo. For these two reasons, which I have shown to exist also in the case of Ascidians, we must, to be consistent, regard the chorda as a mesodermal organ.

Seeliger ('85), Davidoff ('89), and Samassa ('94) all state that the first equatorial plane of cleavage in the ascidian egg separates the two primary germ layers. According to my definition of the primary germ layers in Ciona, this is not true, for several of the cells composing the chorda-mesenchyme ring (included by me in the primary endoderm) are derived from the four ventral cells, which according to their view are exclusively ectodermal. The statement that the first equatorial plane of cleavage separates the two primary germ layers is equally untenable, if tested by the definition of primary germ layers accepted by the authors mentioned; for they include in the primary endoderm the *entire mesoderm*, which I have shown to be derived *chiefly* from the four ventral cells, which produce the definitive *ectoderm*.

B. The Cœlom Theory.

The brothers Hertwig ('81) divided the higher Metazoa into two groups according as the body cavity arises by a pair of outpocketings of the primary endoderm enclosing an *enterocœl* between visceral and parietal mesoderm layers, or by a simple splitting or moving apart of cells in a solid mass of mesoderm, which is then said to enclose a *schizocœl*. The Chordates were unhesitatingly placed by them among the Enterocœlians, and Amphioxus was cited as a typical example. The Tunicates were thus classed as Enterocœlians, though no one had ever observed in their ontogeny the formation of an enterocœl. Van Beneden et Julin ('86) supplied the lack by their studies on Clavelina; but considerable doubt has been thrown on the accuracy of their observations by the independent researches of Seeliger ('85) upon an undetermined species of the same genus, and by those of Davidoff ('91) upon the identical species studied by Van Beneden et Julin. Neither Seeliger nor Davidoff could detect a trace of enterocœl formation in the ontogeny of Clavelina, and Davidoff was equally unsuccessful in finding an enterocœl in Distaplia. My own observations on Ciona are entirely in agreement with those of Seeliger and Davidoff on this point. Van Beneden et Julin, notwithstanding their belief that an enterocœl is formed in Ascidians, as well as in Amphioxus, rejected the classification of the brothers Hertwig on other grounds.

Lwoff ('94) has recently shown that in Amphioxus the cavities enclosed by outpocketings of the wall of the gastral cavity are evanescent structures, and have nothing to do with the subsequently formed body cavity, which, as in all Vertebrates, arises by a wandering apart of mesoderm cells. He therefore concludes "*dass ein wahrer Enterocoelier unter allen Chordaten nicht existirt.*"

I am not able to criticise Lwoff's conclusion from the vantage ground of personal investigation of Amphioxus, but his account bears internal evidence of careful and exact observation. He calls attention to a fact, shown by his figures, that, when the mesodermal pouches arise, spindles, if any are present in the mesoderm cells, invariably stand vertically to the evaginated layer of cells, foreshadowing an arrangement of the daughter cells in two layers. This is exactly the position which the spindles take during gastrulation in the mesenchyme cells of Ciona, but in no other cells of the embryo. The form of division in the mesoderm cells of Amphioxus at the period mentioned tends to obliterate the lumen of the mesodermal pouches, a result which, as Lwoff's figures show, actually comes about. A body cavity is formed only secondarily by the moving apart of the mesoderm cells which are arranged in solid masses, the protovertebræ.

Davidoff ('91) likewise observed in the case of the compound Ascidian, Distaplia, that spindles stand vertically in the cells which give rise to the mesoderm at the time of the separation of the middle germ layer. He believes that the Tunicates can in no sense be regarded as Enterocœlians, and, further, that the distinction made by the brothers Hertwig between those Metazoa which possess a "mesoderm" and those which possess "mesenchyme" is an artificial and unsound one. With these conclusions I entirely agree.

Regarding Rabl's ('89) distinction between "gastral" and "peristomal" mesoderm, my observations lead me to the same conclusion as has been expressed by Davidoff, "dass das *peristomale* Mesoderm der Ascidien sich im weiteren Verlauf der Entwicklung zum *gastralen* herausbildet, oder dass das *gastrale* Mesoderm ursprünglich *peristomales* Mesoderm ist." O. Hertwig ('92) draws a similar conclusion regarding Rabl's distinction as applied to the Vertebrates.

I should also state that both Lwoff and Wilson ('94) find that the pole mesoderm cells described by Hatschek in the case of Amphioxus do not exist. Certainly nothing of the kind is found among Ascidians. Hence we may conclude that such cells are entirely wanting among Chordates.

C. Ancestry of the Chordates.

To determine the phylogenetic relationship of the Chordates to the other groups of Metazoa is a very difficult problem. Various solutions of it have been offered, but none is very generally accepted among zoölogists. The group is sharply marked off from all others by the possession of certain peculiar characters, such as the chorda, gill slits, and hypophysis. Among the higher Metazoa the Chordates seem to have no *near* relatives.

An ingenious suggestion, which has gained considerable currency, is that a chordate is homologous with an annelid whose dorsal and ventral surfaces are reversed. This "annelid hypothesis" has been ably advocated by Dohrn ('75 and '82–'91) and Eisig ('87). An extensive adverse criticism of the hypothesis has been made by Brooks ('93). Is any light thrown on the question by the ontogenetic history of Tunicates? The evidence from that source seems to me chiefly negative. Recent studies of the embryology of Annelids and Mollusks show a truly marvellous correspondence between the developmental processes in these two groups; it is even possible to refer back particular organs in both to homologous blastomeres, and to trace their differentiation through unmistakably similar processes. No doubt is left in the mind as to the close phylogenetic relationship of Annelids and Mollusks. The embryology of Chordates, however, follows an altogether different course, and is as unlike that of Annelids as the adult forms are different.

It is possible that we must go as far down in the animal scale as the Cœlenterates to find an ancestor *common* to the Chordates and any other group of the higher Metazoa. The embryology of Tunicates seems to me to support this view.

Brooks ('93) has shown good reason for believing that all the principal groups of Metazoa arose as small, permanently pelagic forms, such as we find represented to-day, in a somewhat modified form, by Appendicularia in the case of Chordates.

Amphioxus, because of its adaptation to life on the bottom, has probably undergone considerable modification from the ancestral type. For example, the chorda has been extended forward to the extreme anterior end of the body to admit of the animal's burrowing in the sand; a marked asymmetry of the body has also arisen, and its size has doubtless greatly increased, calling for a metameric arrangement of its organs.

The ascidian tadpole, too, has probably been somewhat modified by a great shortening of the free-swimming (ancestral) period of its existence; but here the changes have probably been restricted to a *suppression* of certain processes or organs, so that those which remain are more certainly ancestral than those which occur even in Amphioxus. The *post-larval* history of Ascidians clearly exhibits a process of degeneration, which of course is wholly cœnogenetic.

VIII. CONCLUSIONS.

1. In the maturation of the ascidian egg the polar globules arise at the *vegetative* pole, i. e. in the future *endodermal* portion of the egg.

2. The archoplasms (attraction spheres) of the first cleavage spindle, and consequently of all subsequent spindles of the fertilized ascidian egg, are derived exclusively from the spermatazoön.

3. The archoplasm (attraction sphere) is not an organ of heredity, since in sexual reproduction it is frequently derived from one parent only.

4. Cleavage in the ascidian egg is bilateral from the very beginning. The course of cleavage is less variable in the egg of Ciona than in that of Amphioxus or the Vertebrates, and is predetermined by the internal constitution of the unsegmented egg.

5. The first equatorial plane of cleavage does not separate completely the two primary germ layers, though it does separate definitive endoderm from definitive ectoderm.

6. The fundaments of the principal organs are arranged in zones around the chief axis of the egg.

7. The nervous system and the longitudinal musculature of the larva are derived from a *common fundament*, which is a (neuro-muscular) ring of cells encircling the margin of the blastopore. This ring of cells must be regarded as a part of the primary ectoderm.

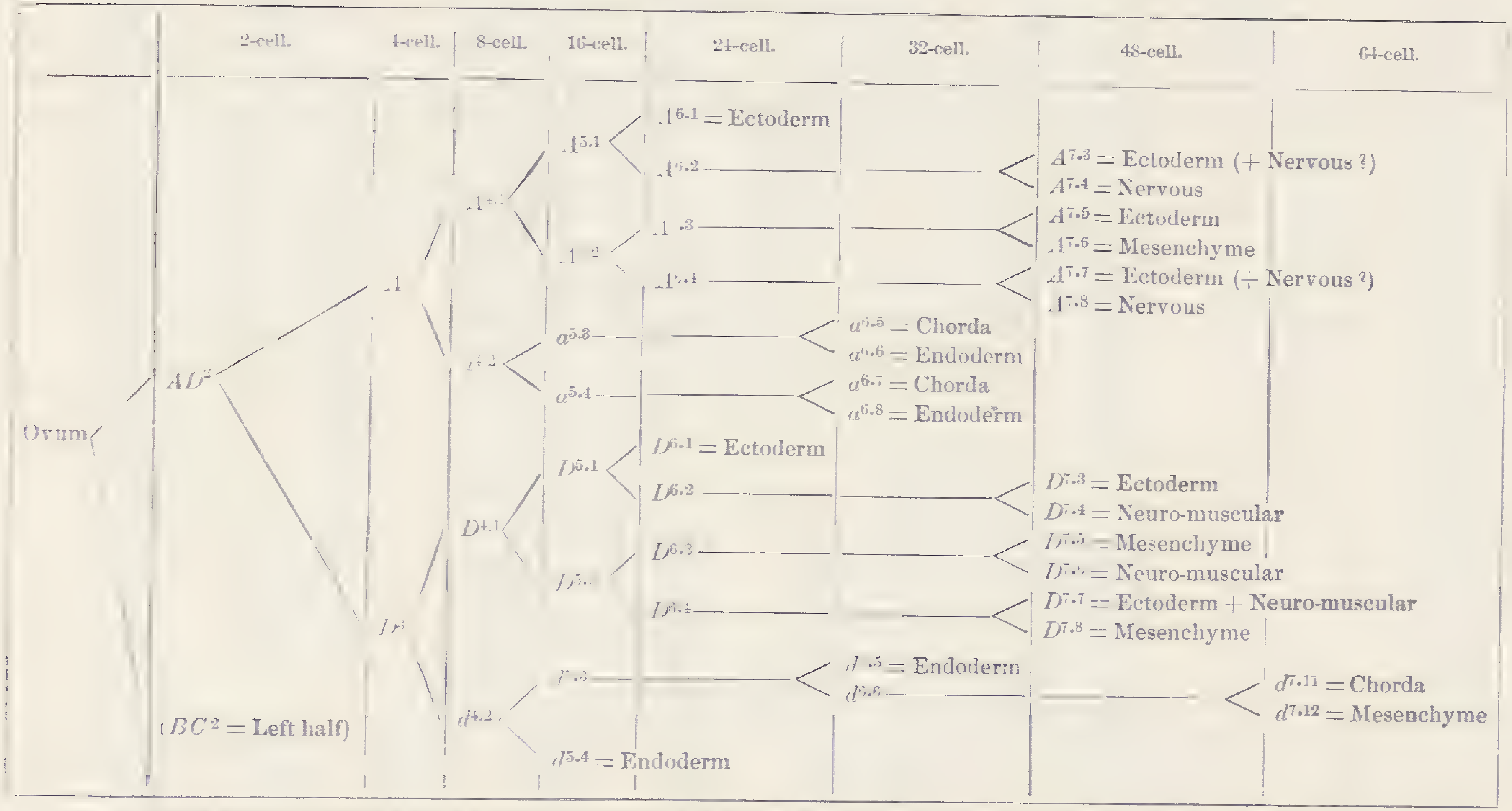
8. The chorda and mesenchyme (or trunk mesoderm) are derived from another ring of cells lying just within the margin of the blastopore. This ring of cells is to be regarded as a part of the primary endoderm.

9. The mesoderm of Ascidians is therefore derived in part from the primary ectoderm, and in part from the primary endoderm. It is formed exclusively by cells of the two rings already mentioned, one of which belongs to each of the two primary germ layers. Recent careful observations indicate that likewise in Amphioxus and the Vertebrates the mesoderm is derived from both primary germ layers.

10. The longitudinal musculature of the Ascidian tadpole is homologous with that of Amphioxus; the mesenchyme of the Ascidian, with the mesoderm lateral to the muscle plates in Amphioxus.

11. The chorda should be regarded as a mesodermal organ.

IX. TABLE OF CELL LINEAGE OF CIONA.


2-cell.
4-cell.
8-cell.
16-cell.
24-cell.
32-cell.
48-cell.
64-cell.
Ovum
AD^2
$(BC^2$ = Left half)
A^3
D^3
$A^{4.1}$
$a^{4.2}$
$D^{4.1}$
$d^{4.2}$
$A^{5.1}$
$A^{5.2}$
$a^{5.3}$
$a^{5.4}$
$D^{5.1}$
$D^{5.2}$
$d^{5.3}$
$d^{5.4}$ = Endoderm
$A^{6.1}$ = Ectoderm
$A^{6.2}$
$A^{6.3}$
$A^{6.4}$
$D^{6.1}$ = Ectoderm
$D^{6.2}$
$D^{6.3}$
$D^{6.4}$
$a^{6.5}$ = Chorda
$a^{6.6}$ = Endoderm
$a^{6.7}$ = Chorda
$a^{6.8}$ = Endoderm
$d^{6.5}$ = Endoderm
$d^{6.6}$
$A^{7.3}$ = Ectoderm (+ Nervous ?)
$A^{7.4}$ = Nervous
$A^{7.5}$ = Ectoderm
$A^{7.6}$ = Mesenchyme
$A^{7.7}$ = Ectoderm (+ Nervous ?)
$A^{7.8}$ = Nervous
$D^{7.3}$ = Ectoderm
$D^{7.4}$ = Neuro-muscular
$D^{7.5}$ = Mesenchyme
$D^{7.6}$ = Neuro-muscular
$D^{7.7}$ = Ectoderm + Neuro-muscular
$D^{7.8}$ = Mesenchyme
$d^{7.11}$ = Chorda
$d^{7.12}$ = Mesenchyme

Note.—In this Table the lineage is given for only the right half of the egg in the 4-cell and subsequent stages. The complete lineage for the *left* half of the egg may be expressed by substituting *B* for *A*, and *C* for *D* in the Table.

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'95. Maturation, Fertilization, and Polarity in the Echinoderm Egg. New Light on the "Quadrille of the Centers." Journ. Morph., Vol. X. pp. 319–342, 7 Figs.

EXPLANATION OF PLATES.

All Figures were drawn with the aid of the Abbé camera lucida. The magnification is stated for each plate separately. The egg membranes have not been represented in any of the Figures. Arrows in the Figures connect sister cells, i. e. cells which have arisen by division of a common mother cell. For an explanation of the system of nomenclature employed, see page 226.

In many of the Figures, only the cells of one half of the embryo (usually the right, that is, quadrants A and D) have been lettered. The reader will be able readily to supply the deficiencies for the cells of the other half, since they are almost perfectly symmetrical in position with those that are lettered. Corresponding to each cell on one side of the median plane (in quadrant A or D) will be found a cell similarly situated on the *other* side of the median plane (in quadrant B or C); this cell should receive the same exponents as its mate, and either the letter B or C according as that mate is lettered A or D. Thus, the mate of $A^{7.2}$ in Figure 58 (Plate X.) is the cell immediately to the right of it, which would be called $B^{7.2}$; the respective mates of $D^{7.5}$ and $D^{7.6}$ in Figure 57 would be called $C^{7.5}$ and $C^{7.6}$.

In Figure 62 (Plate X.) and all following Figures, the endoderm cells are indicated by *granular nuclei* in a cell body that is left without tint or stippling; the chorda cells without tint or stippling, and with the outlines only of nuclei; the mesenchyme cells by a flat tint; the ring of neuro-muscular cells by stippling of the body of the cell. The ectoderm cells, since they are easily distinguishable from the endoderm cells, have been left, like the latter, without stippling or tint; their nuclei are sometimes drawn true to nature, i. e. with granulations, sometimes in outline only.

ABBREVIATIONS.

ast. ♀ = female astral sphere.
ast. ♂ = male astral sphere.
cd. = chorda.
cl. pol. = polar globule.
cl. pol.$^{I.}$ = first polar globule.
ec'drm. = ectoderm.
en'drm. = endoderm.
ms'chy. = mesenchyme.
mu. = musculature.
n. = nervous system.
pr'nl. = pronucleus.
pr'nl. ♀ = female pronucleus.
pr'nl. ♂ = male pronucleus.

PLATE I.

All Figures represent sections; magnification, 560 diameters. Yolk granules are not represented, except in Fig. 2.

Fig. 1. First maturation spindle, and formation of first polar globule.

Fig. 2. Portion of section through recently impregnated egg. The spermatozoön lies at the centre of a region free from yolk granules.

Fig. 3. Second maturation spindle.

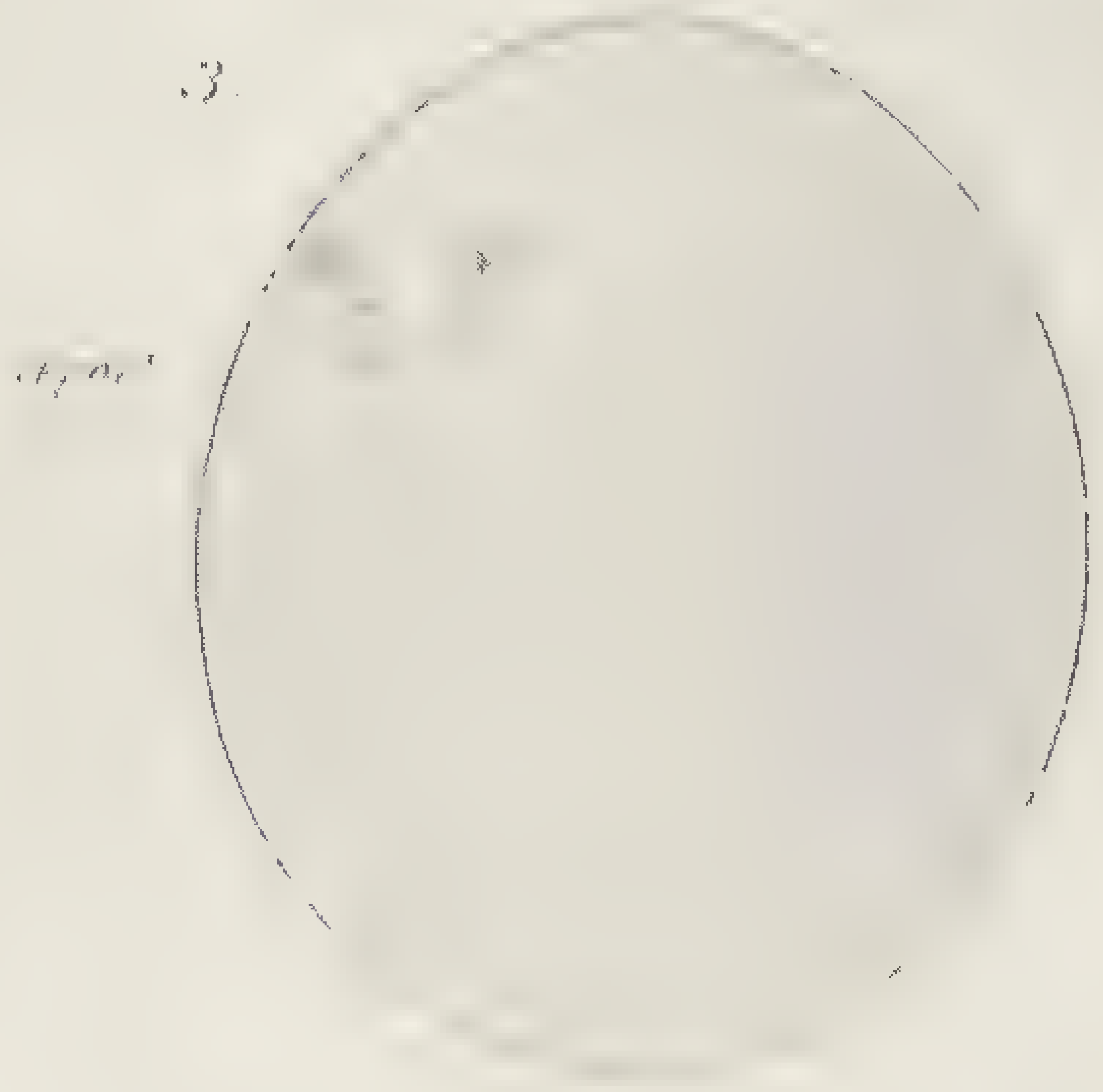
Fig. 4. Section through the same egg in region of male pronucleus.

Fig. 5. Impregnated egg; male and female pronuclei visible in the same section.

Fig. 6. Conjugation of pronuclei, viewed in the direction of the axis of the first cleavage spindle, which is in process of formation. (Cf. Plate III. Fig. 13.)

1.

3.



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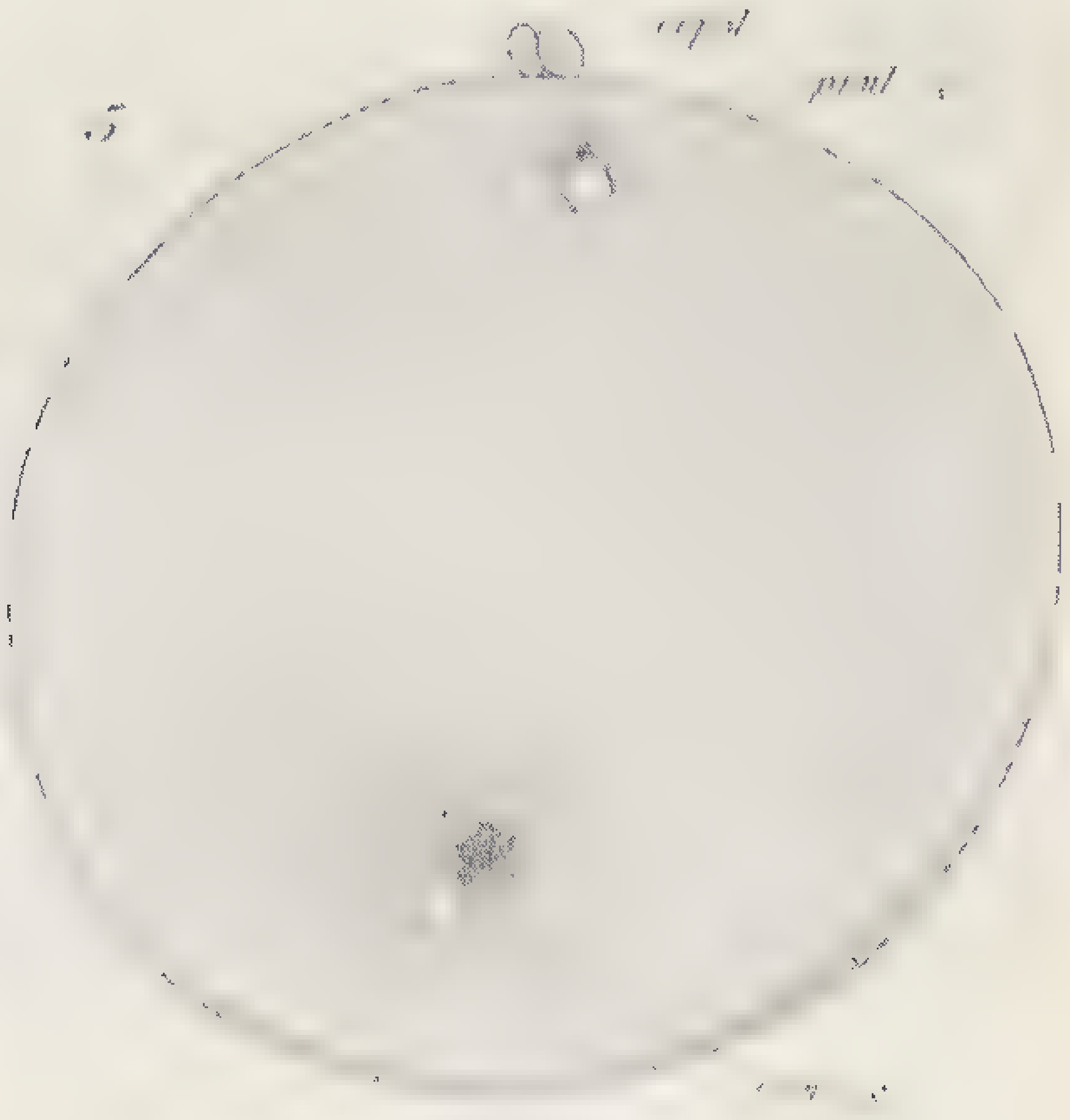


4.

6.

2.

PLATE II.

Figs. 7–10. Four sections through an impregnated egg; magnification, 560 diameters.

Fig. 7. Fourth section of series, showing the male pronucleus and archoplasmic spheres. Compare Figs. 11 and 12.

Fig. 8. Seventh section of series, showing the female pronucleus.

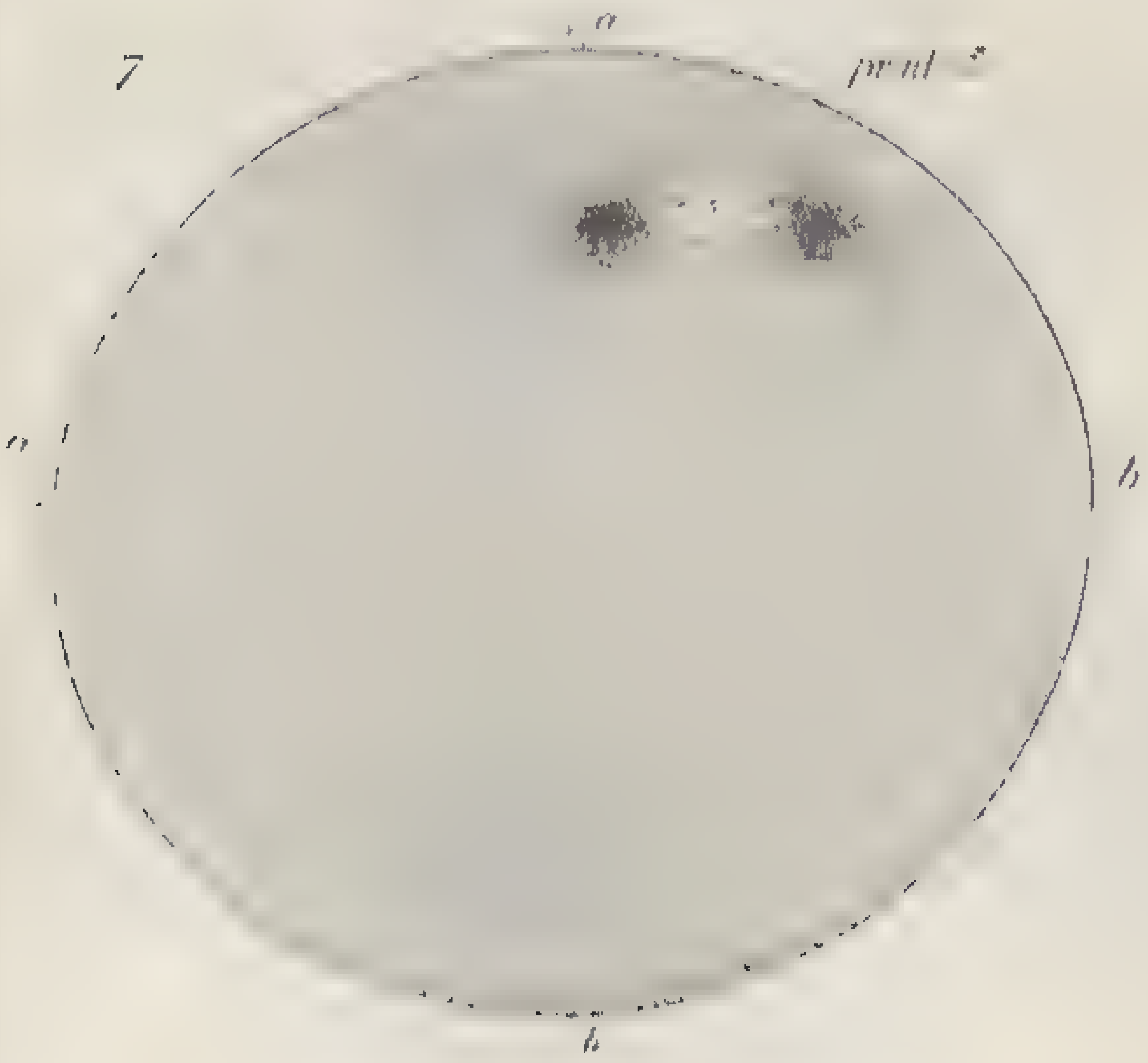
Fig. 9. Tenth section of series, showing the female archoplasm.

Fig. 10. Twelfth section of series, showing polar globules.

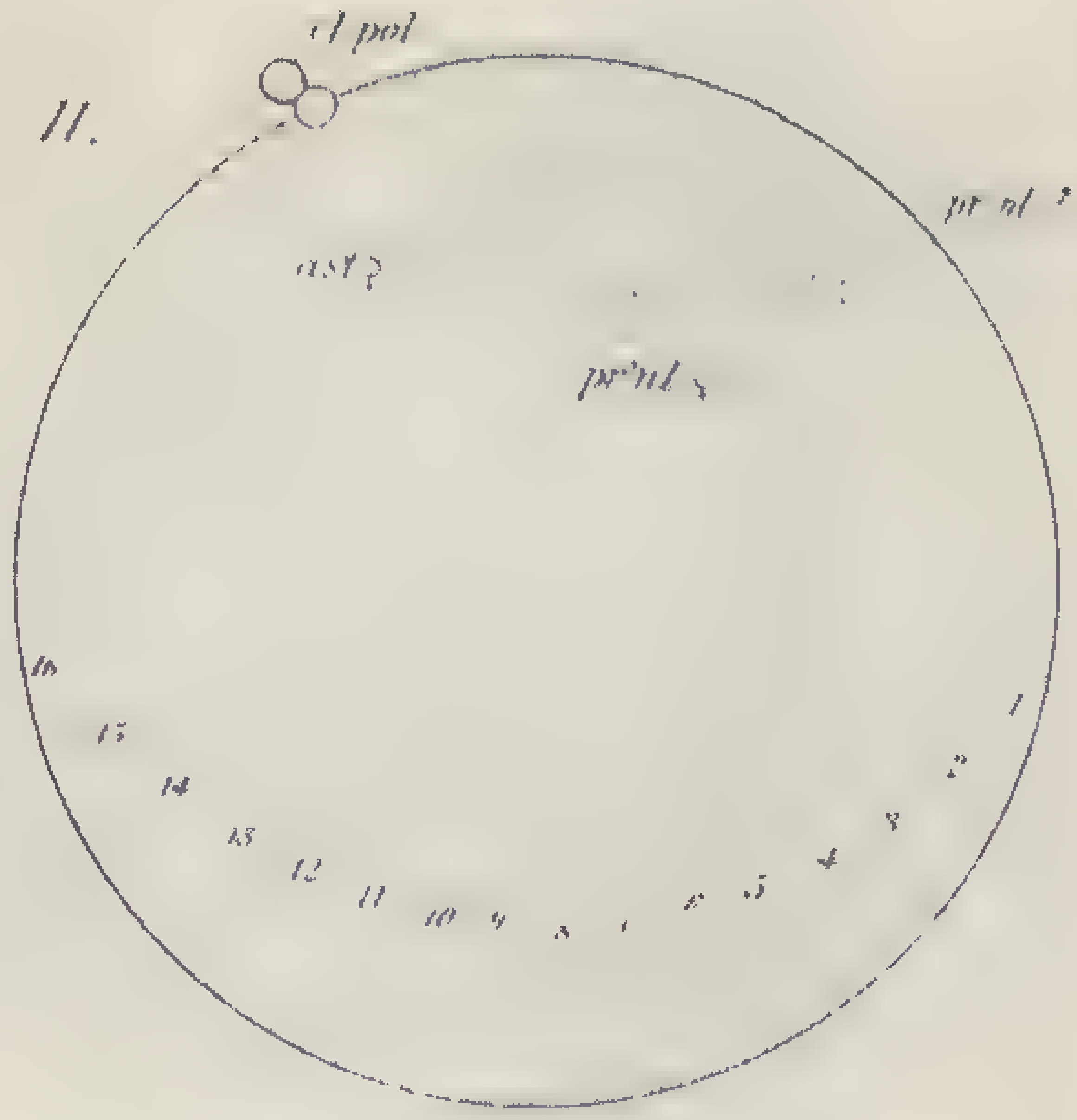
Fig. 11. Graphic reconstruction of the series on a plane, perpendicular to that of Fig. 7, indicated by the line *a b*, Fig. 7.

Fig. 12. A similar reconstruction on a perpendicular plane, the projection of which is the line *a′ b′*, Fig. 7.

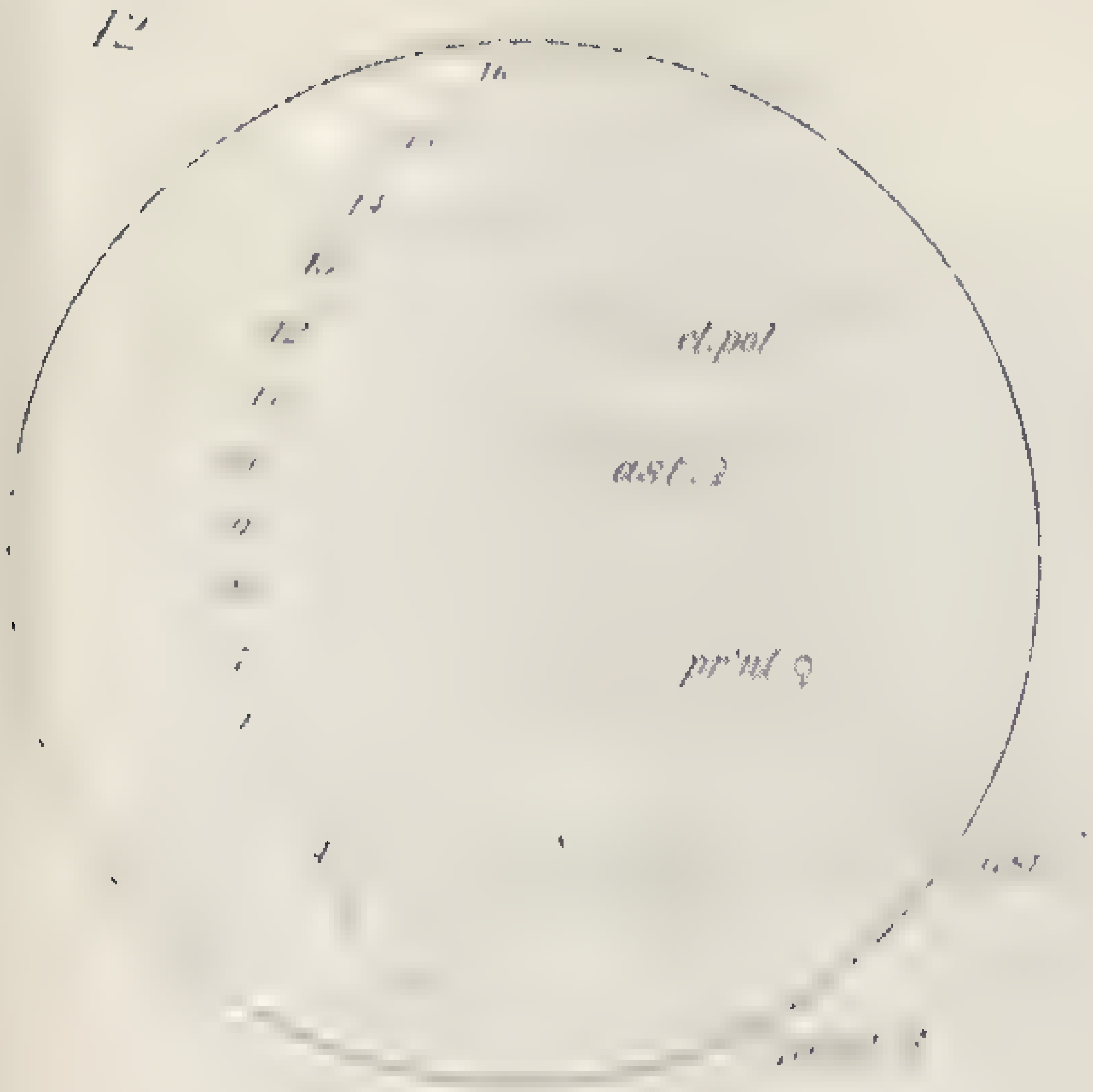
7


a
pr'nl ♂
a
b
b

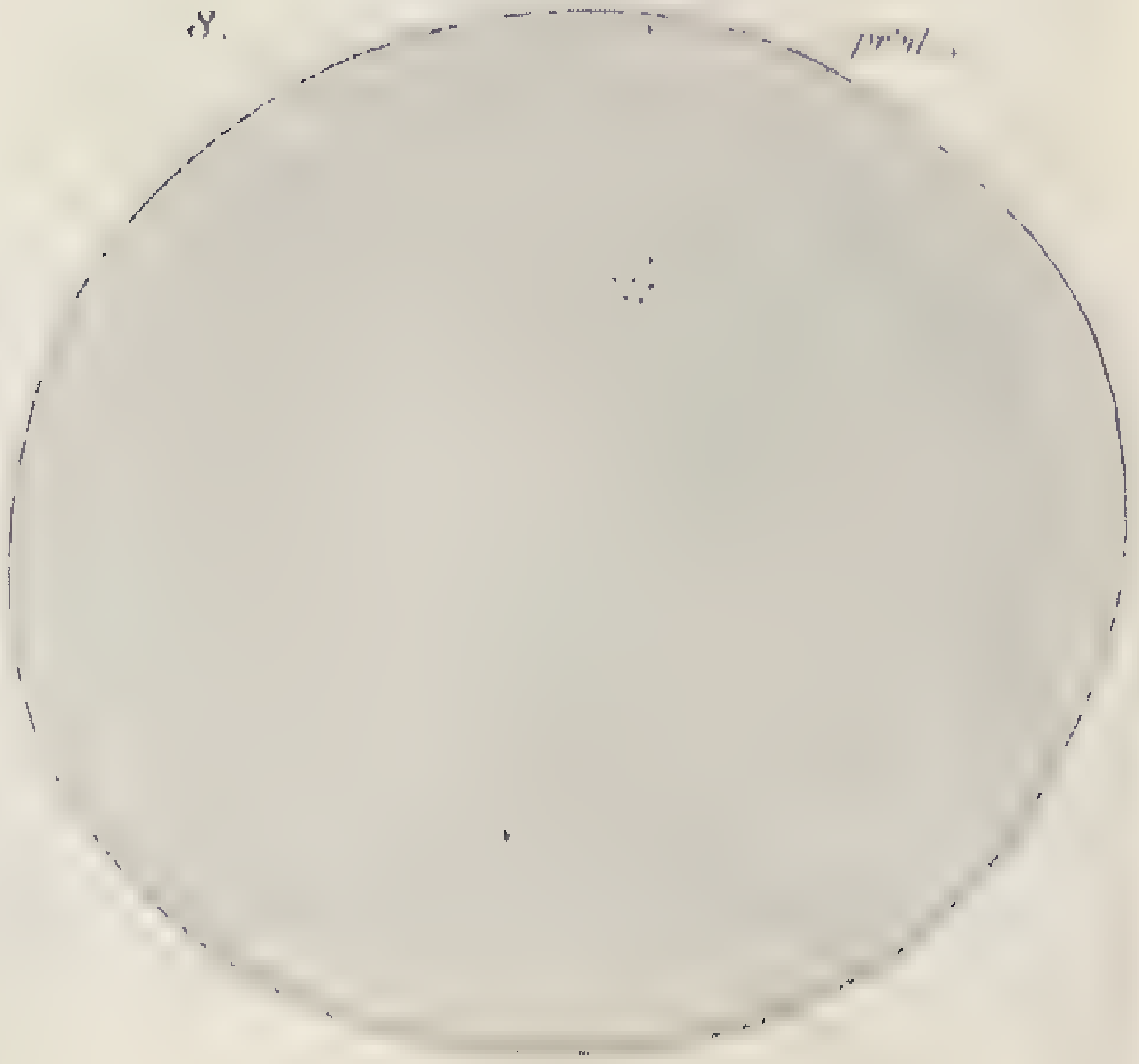
11.


cl.pol
pr'nl ♂
ast ?
pr'nl ♀
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

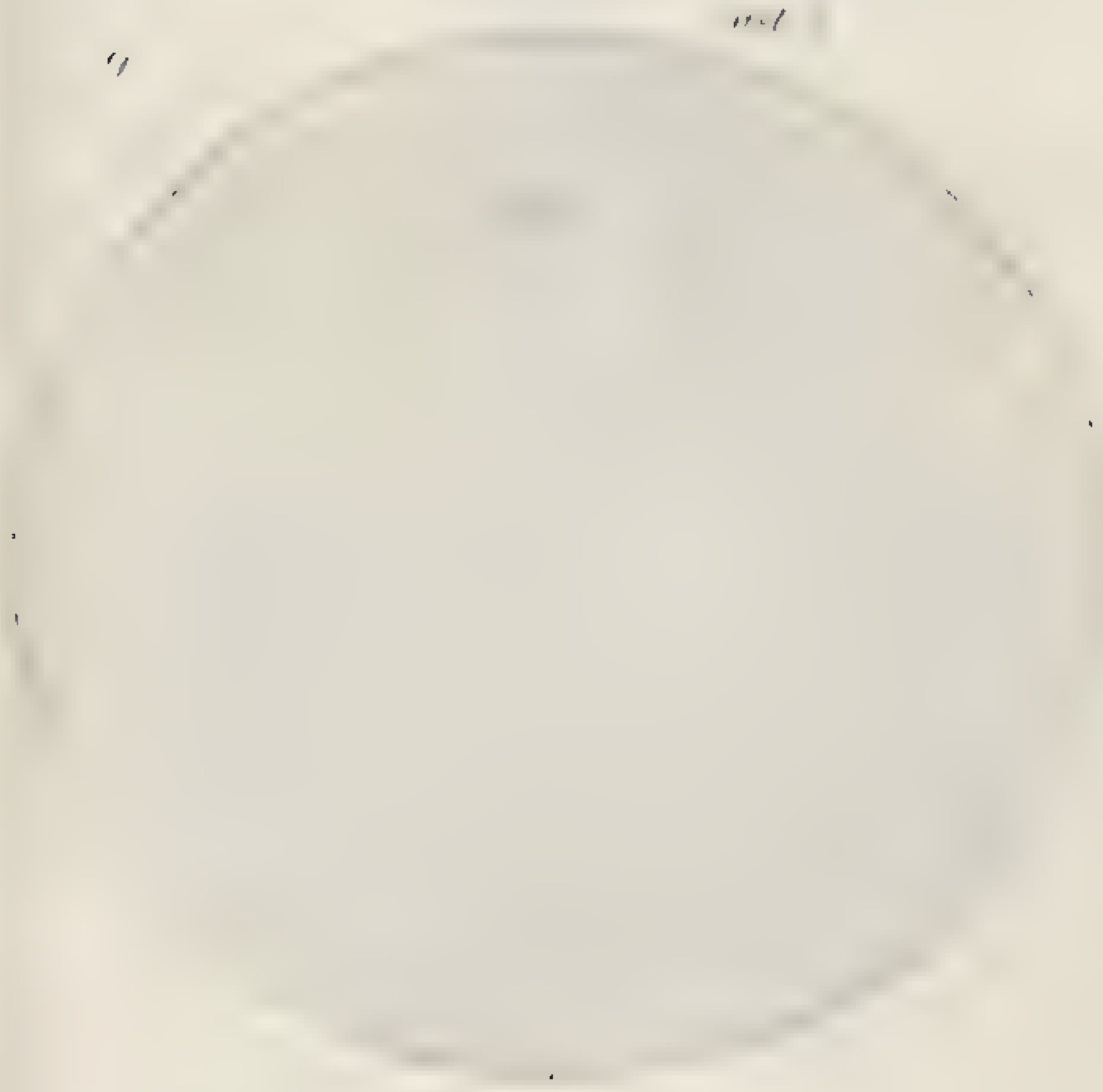
12


16
15
14
13
12
11
10
9
8
7
cl.pol
ast. ?
pr'nl ♀
ast
pr'nl ♂

8.


pr'nl ♀

9


ast ?

10

cl.pol

PLATE III.

All Figures represent sections; magnification, 560 diameters.

Fig. 13. Conjugation of pronuclei, viewed in the direction of the equator of the first cleavage spindle. (Cf. Plate I. Fig. 6.)

Fig. 14. First cleavage spindle.

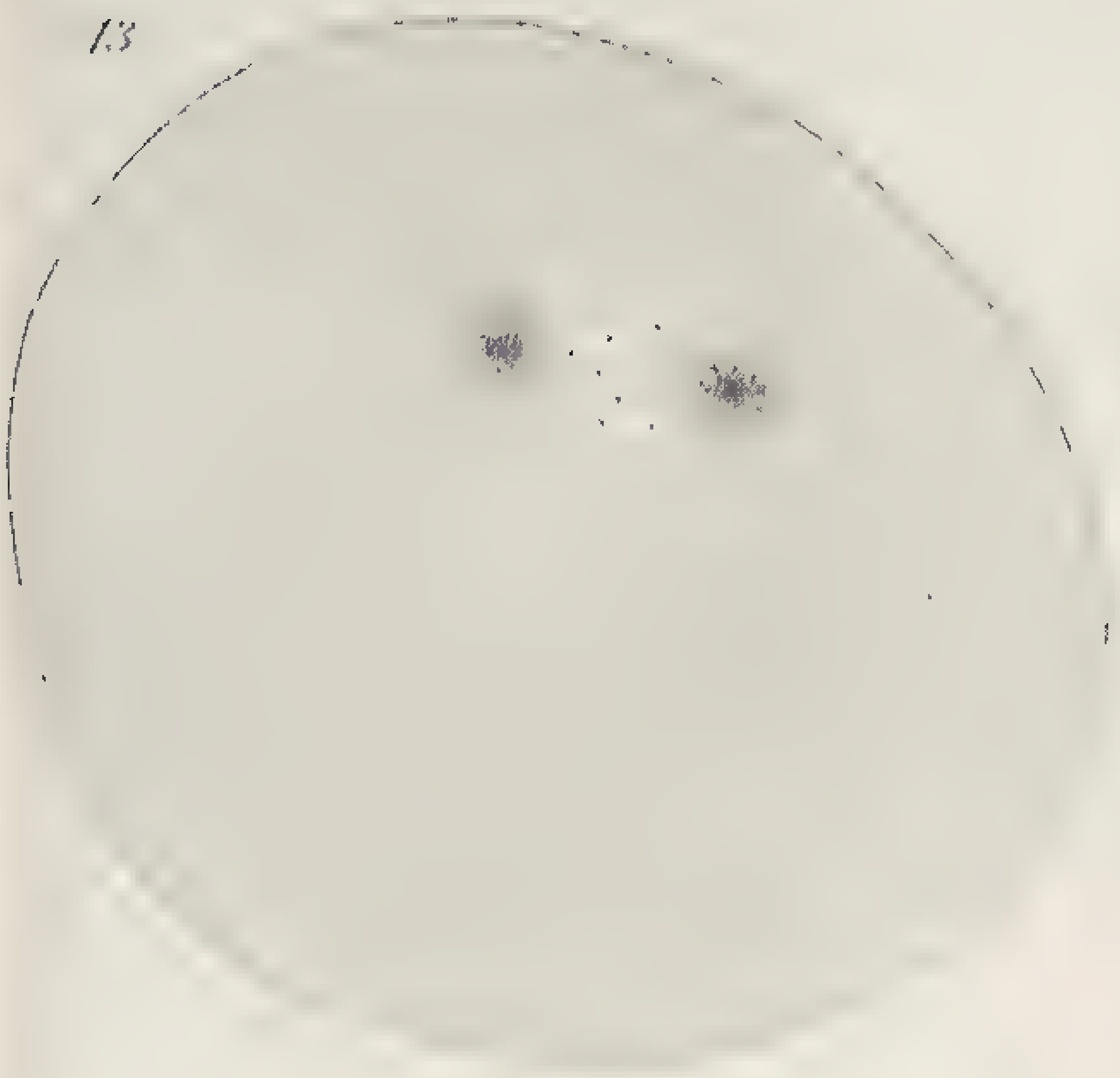
Fig 15. First cleavage nearly completed. Each of the newly formed nuclei is made up of two vesicles as yet incompletely fused.

Fig. 16. Section through one of the cells of a 2-cell stage, parallel to median plane of the embryo.

Fig. 17. Section from the same series as Fig. 16 through one of the two cells, near the median plane of the embryo. x, finely granular protoplasm, which marks the posterior-ventral side of embryo (cf. Plate VIII. Fig. 45, x), and is traceable up to the larval stage.

Fig. 18. Section through an 8-cell stage parallel to median plane of the embryo.

13.



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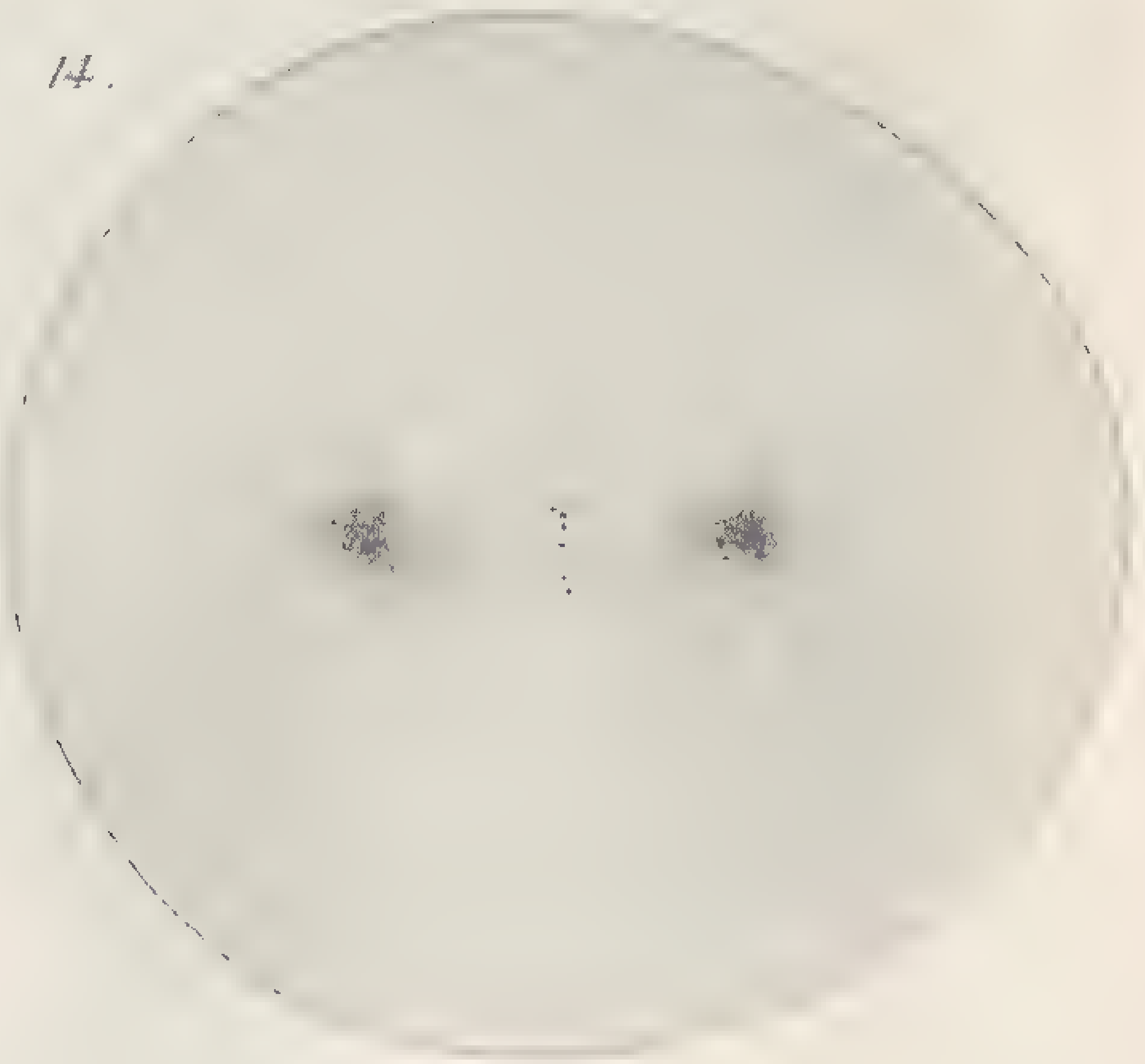

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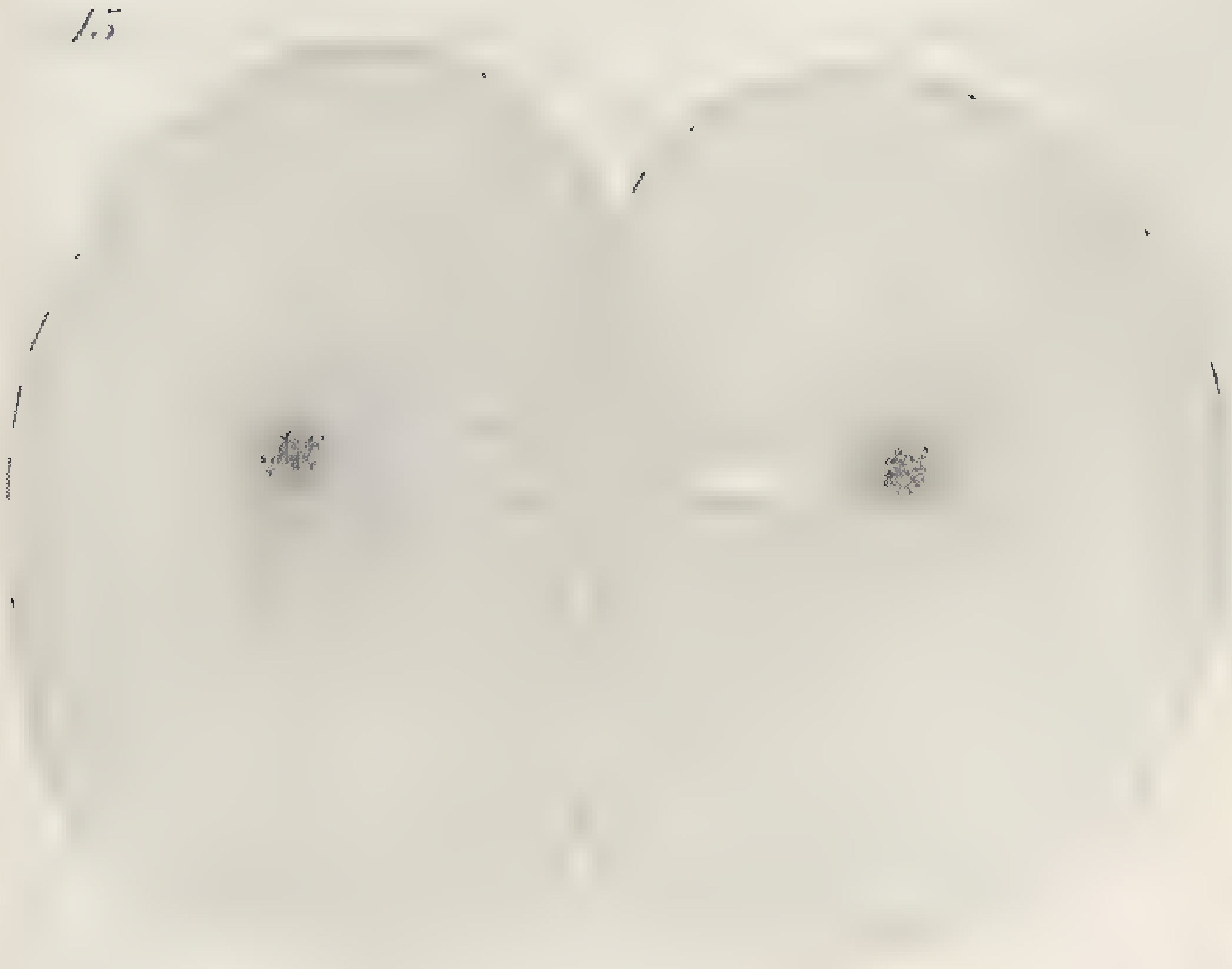

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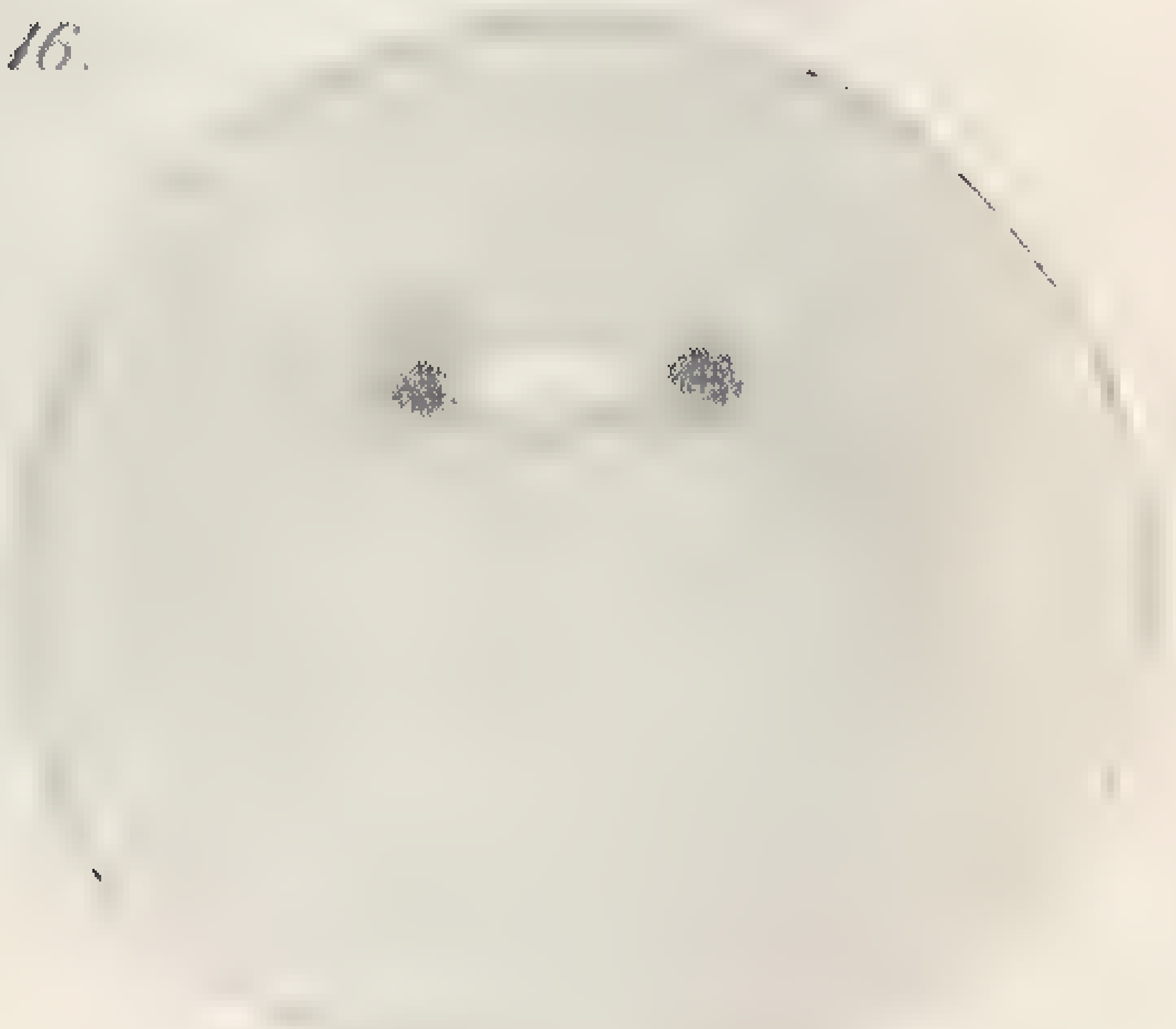


17.

18.

19.

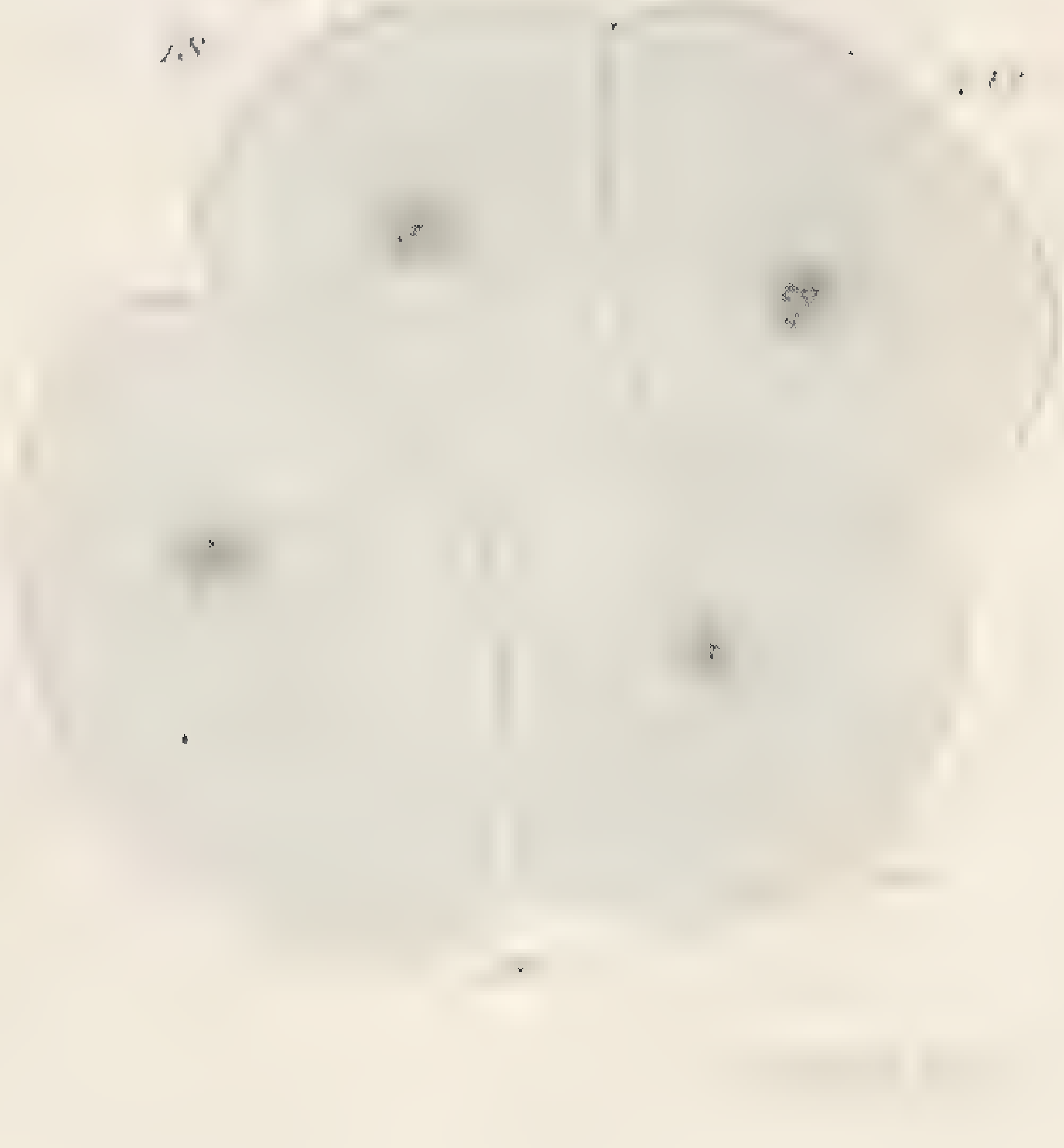
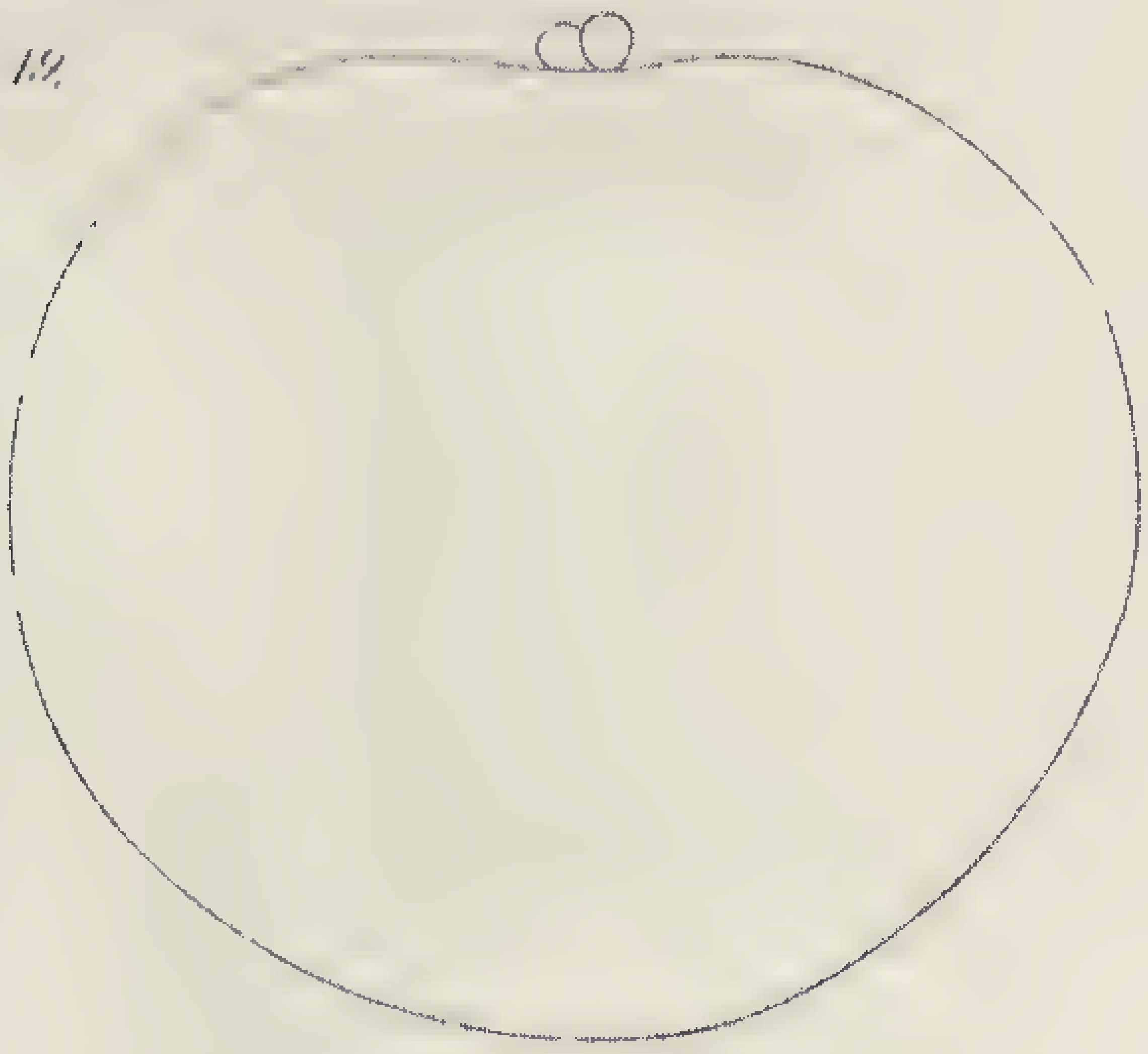


PLATE IV.

Eight successive views of a living egg, seen from the left side; magnification, 315 diameters. Arrows indicate the direction of spindles.

Fig. 19. Matured but unsegmented egg.
Fig. 20. 2-cell stage.
Fig. 21, 4-cell stage approaching.
Fig. 22. 4-cell stage, "resting" condition.
Fig. 23. 8-cell stage, just formed.
Fig. 24. 8-cell stage, nine minutes later.
Fig. 25. 16-cell stage, just formed.
Fig. 26. 16-cell stage, some minutes later.

19.



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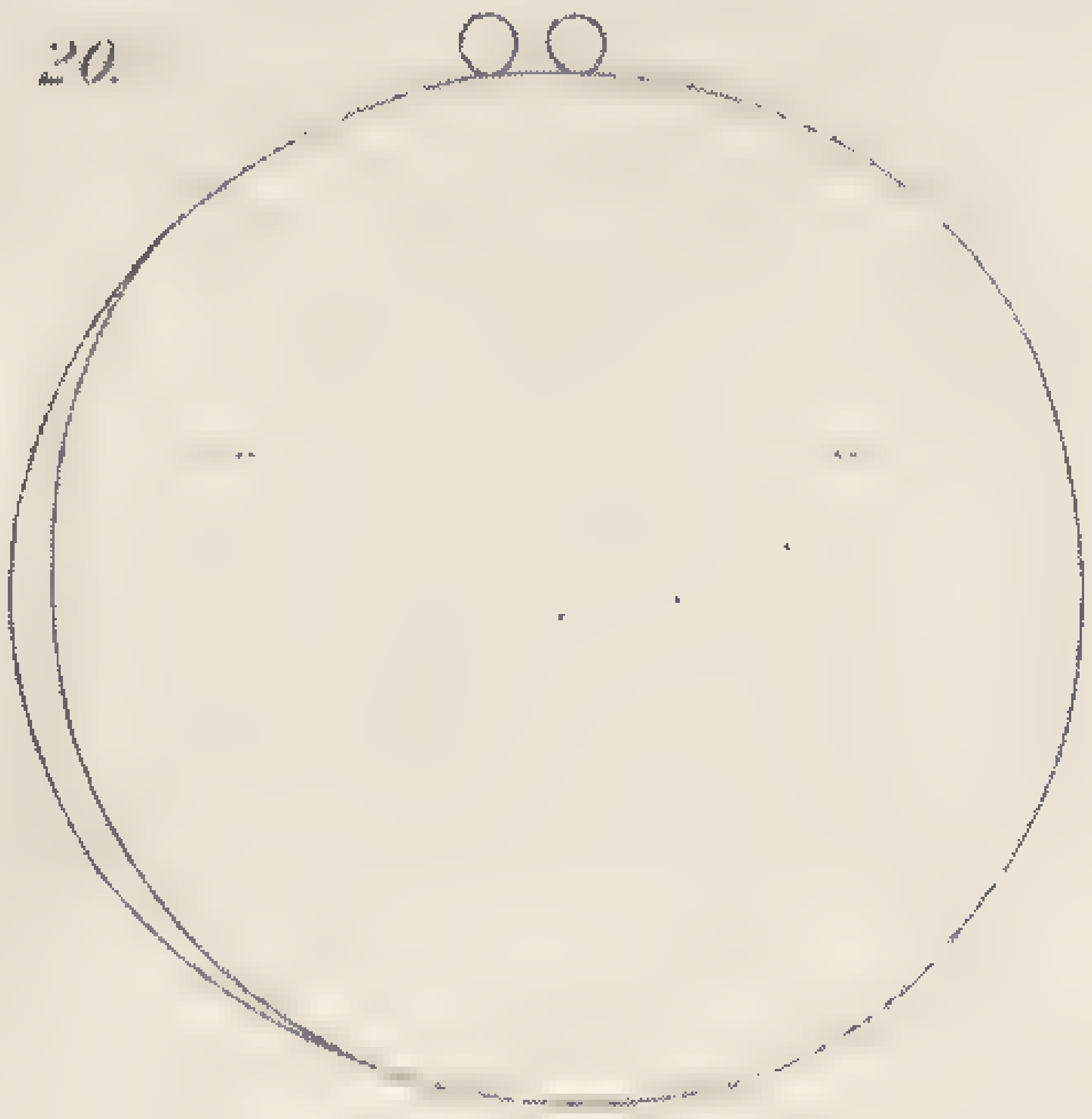

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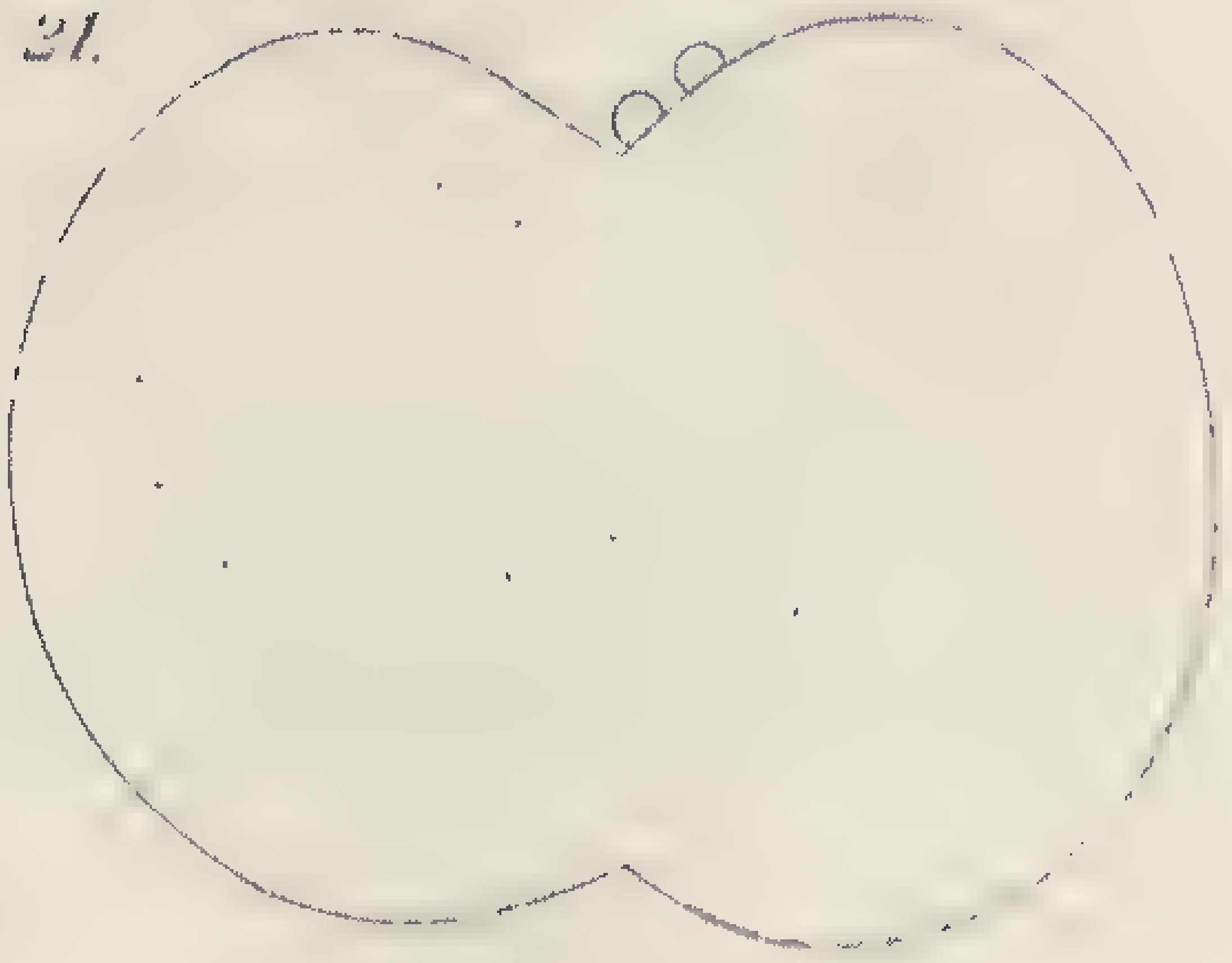

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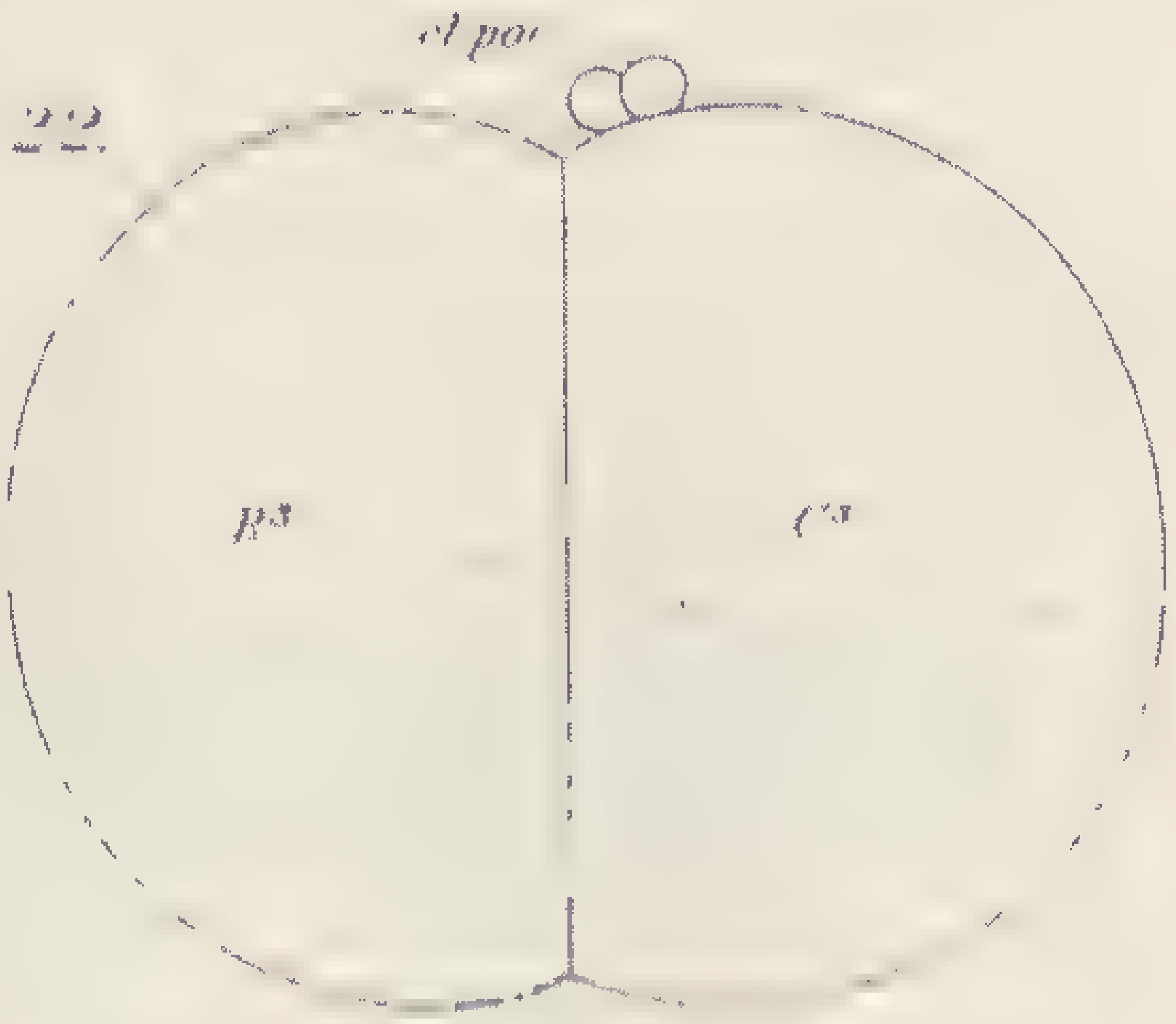

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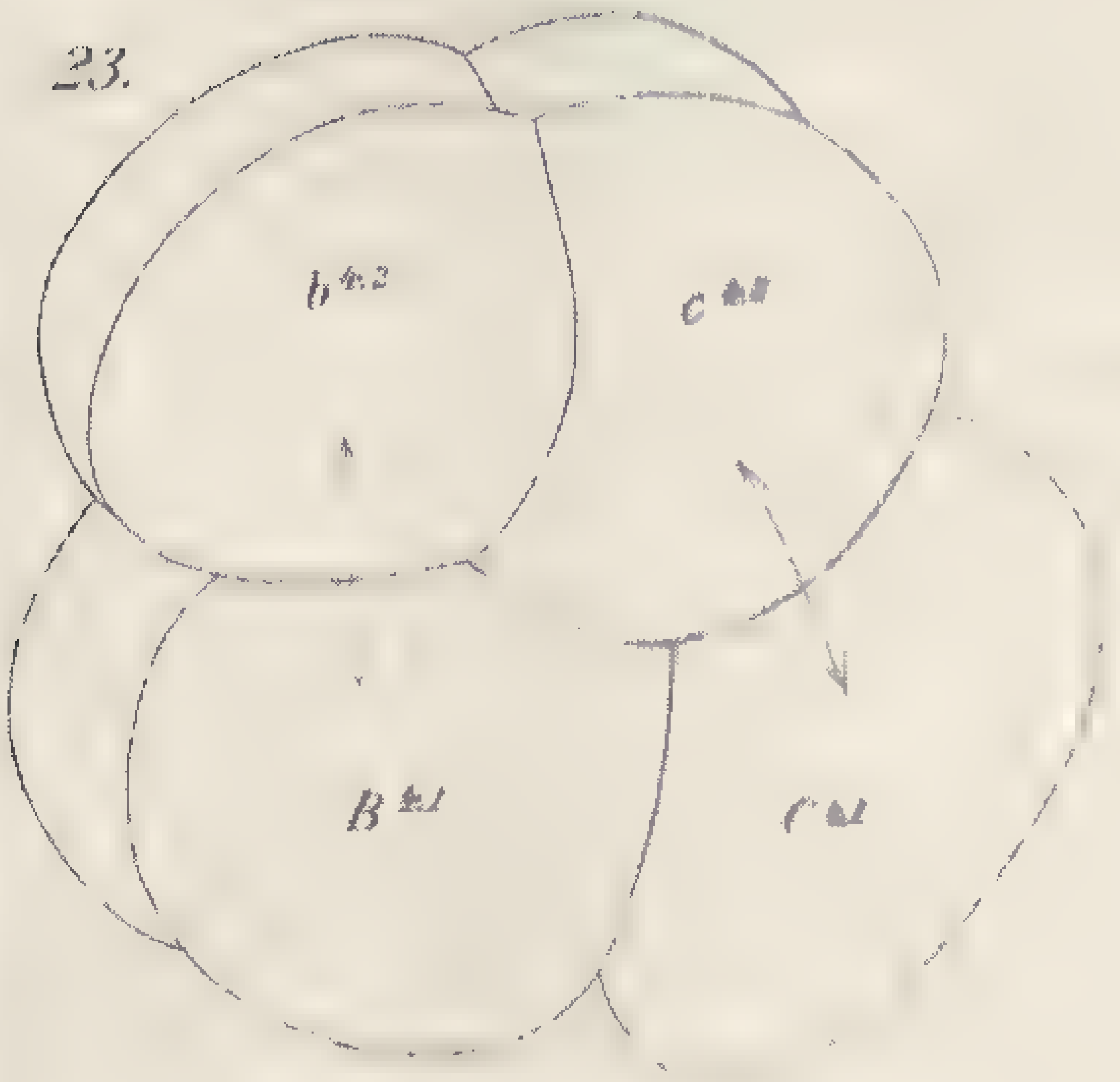
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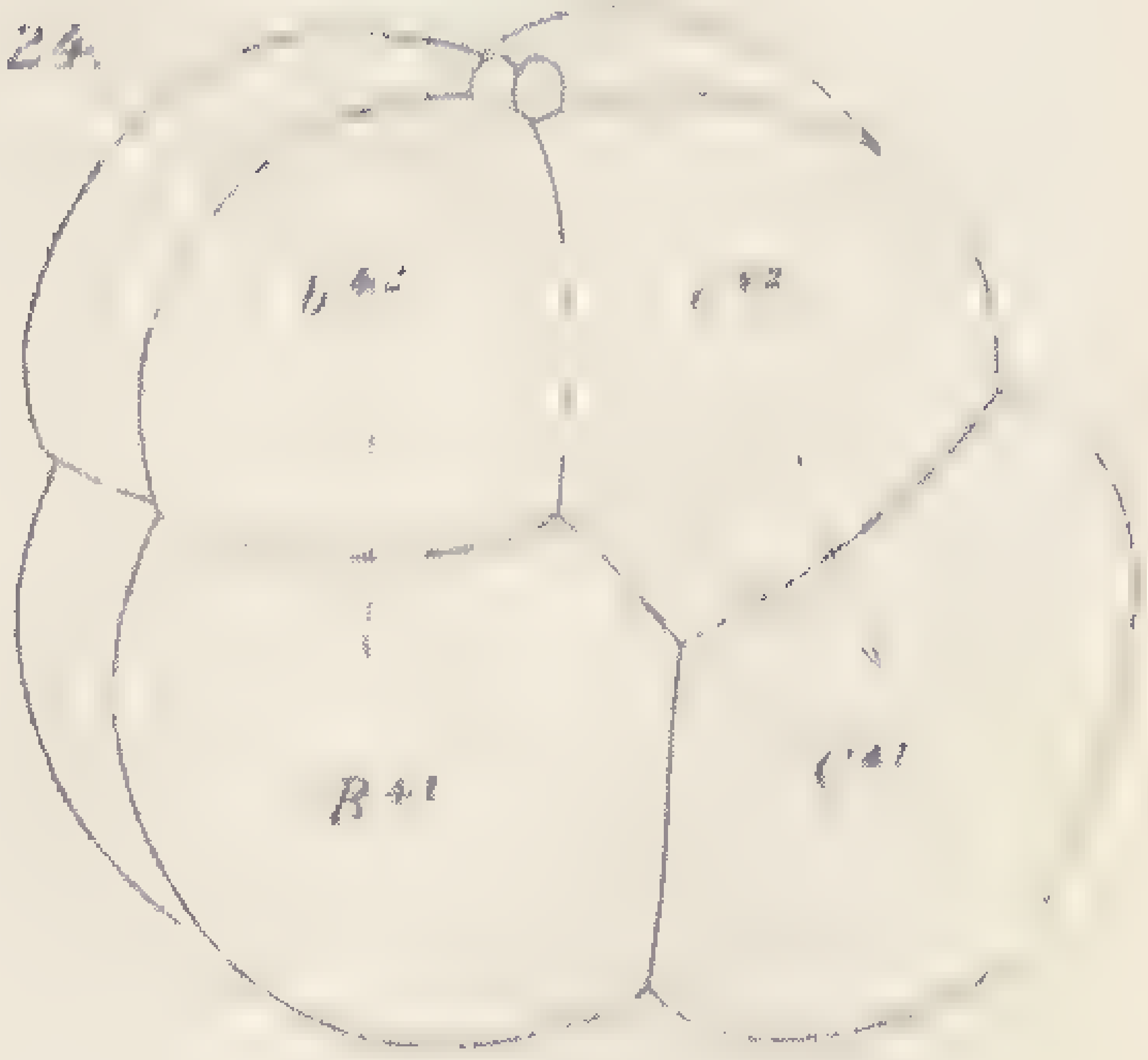
$c^{4.2}$
$B^{4.1}$
$C^{4.1}$

24.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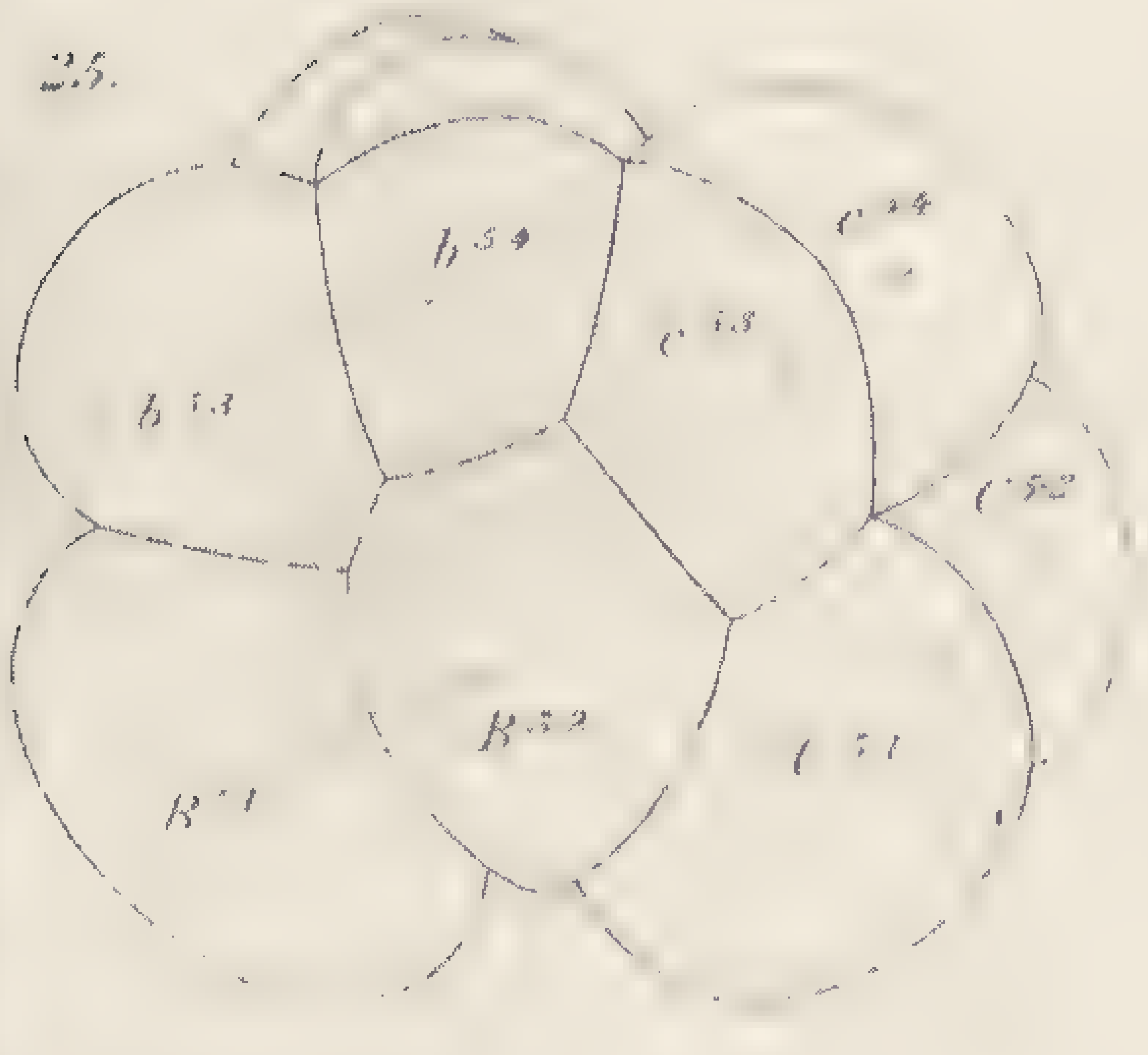
$B^{4.1}$
$C^{4.1}$

25.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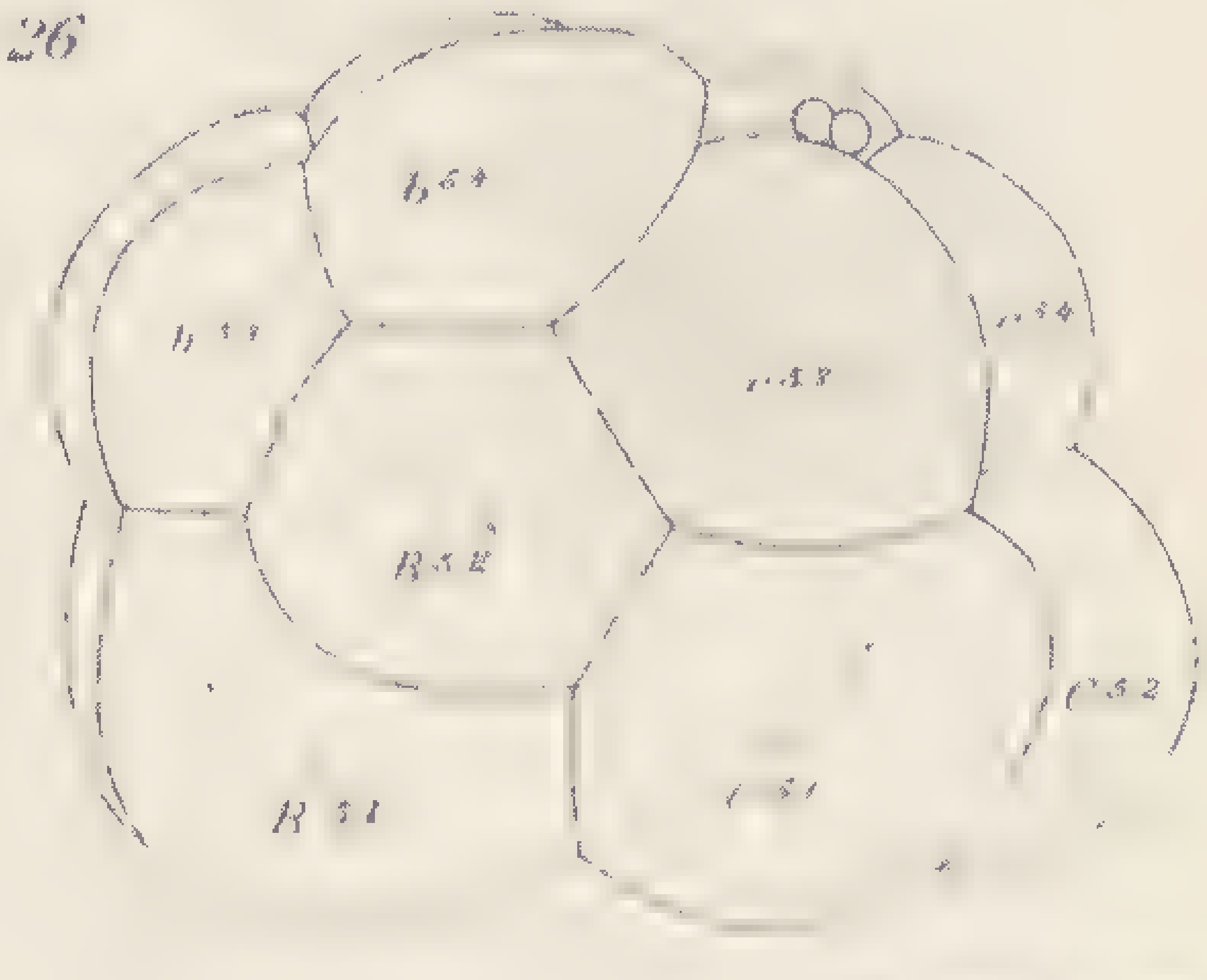
$B^{5.1}$

26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$b^{5.3}$
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$c^{5.3}$
$B^{5.2}$
$C^{5.2}$
$B^{5.1}$
$C^{5.1}$

W.E.C. del. B. Meisel, lith. Boston.

PLATE V.

Six successive views of a living egg, seen from the anterior end; magnification, 315 diameters. Figs. 27–31 show the egg viewed as a transparent object. Fig. 32 is a surface view.

Fig. 27. 2-cell stage, newly formed.
Fig. 28. 2-cell stage, a few minutes later.
Fig. 29. 2-cell stage, a few minutes later still.
Fig. 30. 4-cell stage.
Fig. 31. 8-cell stage, just formed.
Fig. 32. 8-cell stage, a few minutes later.

27

28

29

30

31

32

PLATE VI.

Magnification of all Figures, 315 diameters. Arrows indicate the direction of spindles.

Fig. 33. Later stage of the same egg as that shown in Fig. 32, Plate V. 16-cell stage, just formed.

Fig. 34. The same egg, passing into 24-cell stage.

Figs. 35–38. Ventral aspect of four successive stages of a living egg.

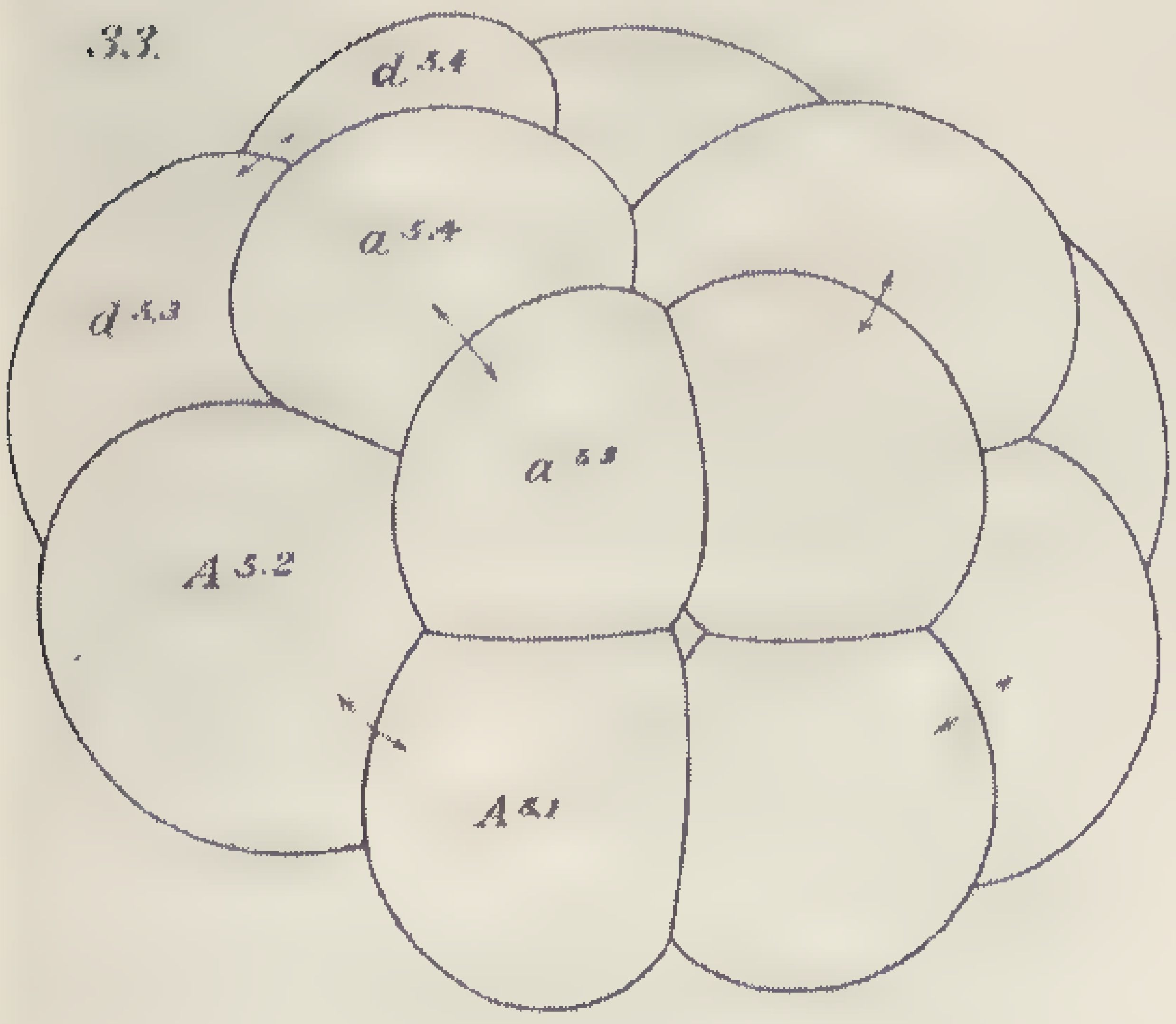
Fig. 35. 4-cell stage.

Fig. 36. 8-cell stage, just formed.

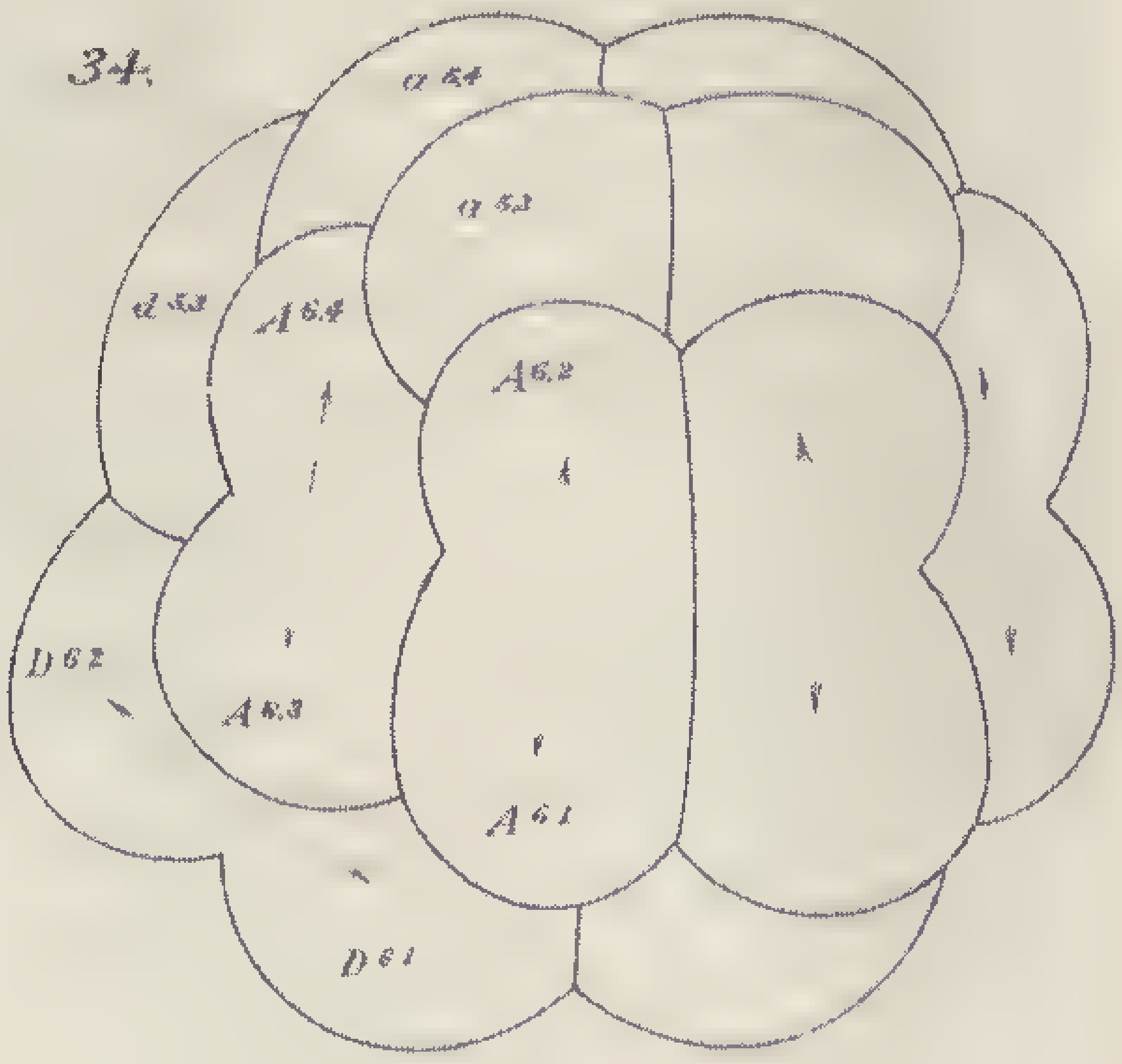
Fig. 37. 8-cell stage, a few minutes later.

Fig. 38. 12-cell stage, just formed.

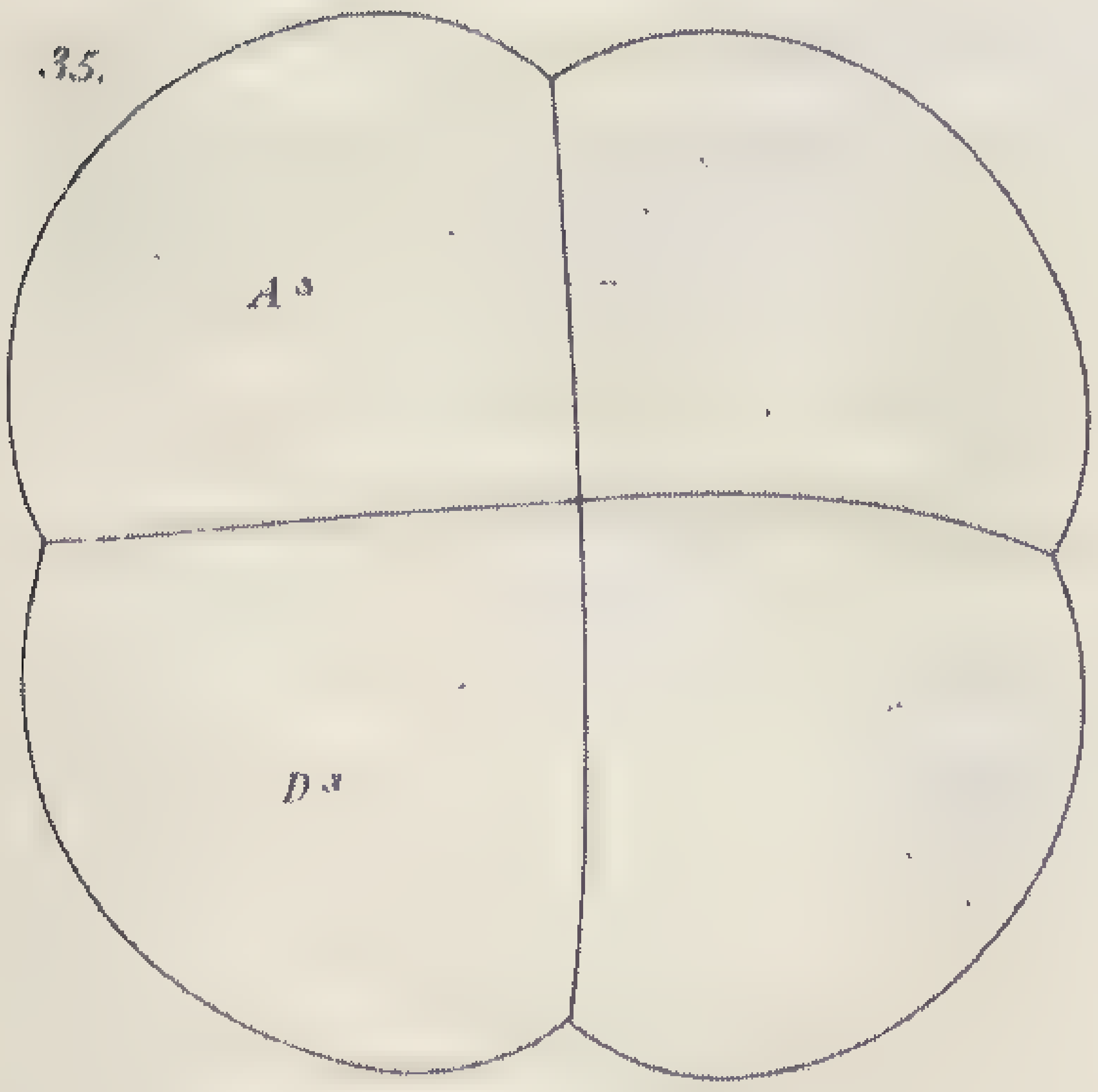
33.


d5.4
a5.4
d5.3
a5.3
A5.2
A5.1

34.


a6.4
a6.3
d6.3
A6.4
A6.2
D6.2
A6.3
A6.1
D6.1

35.



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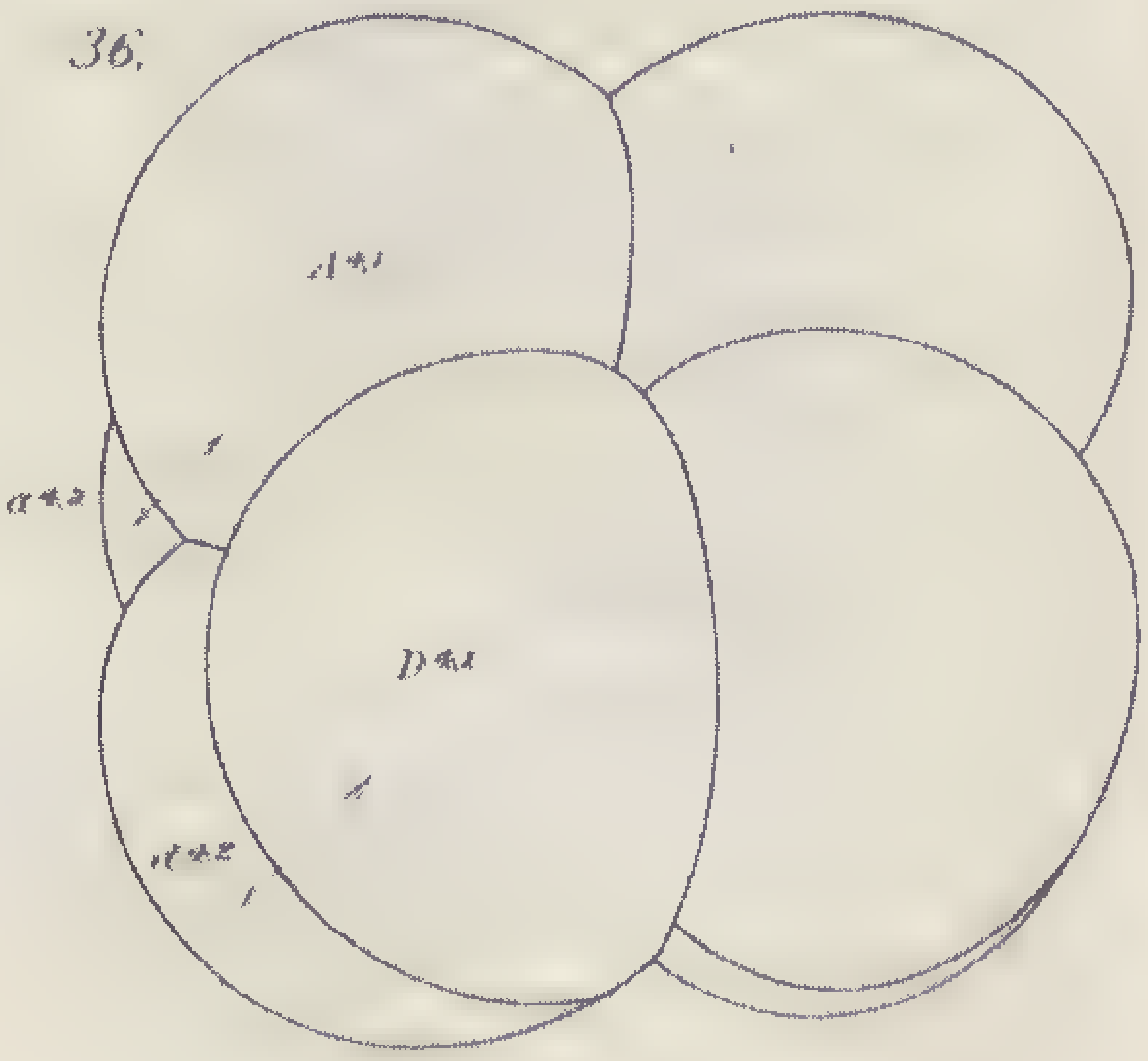
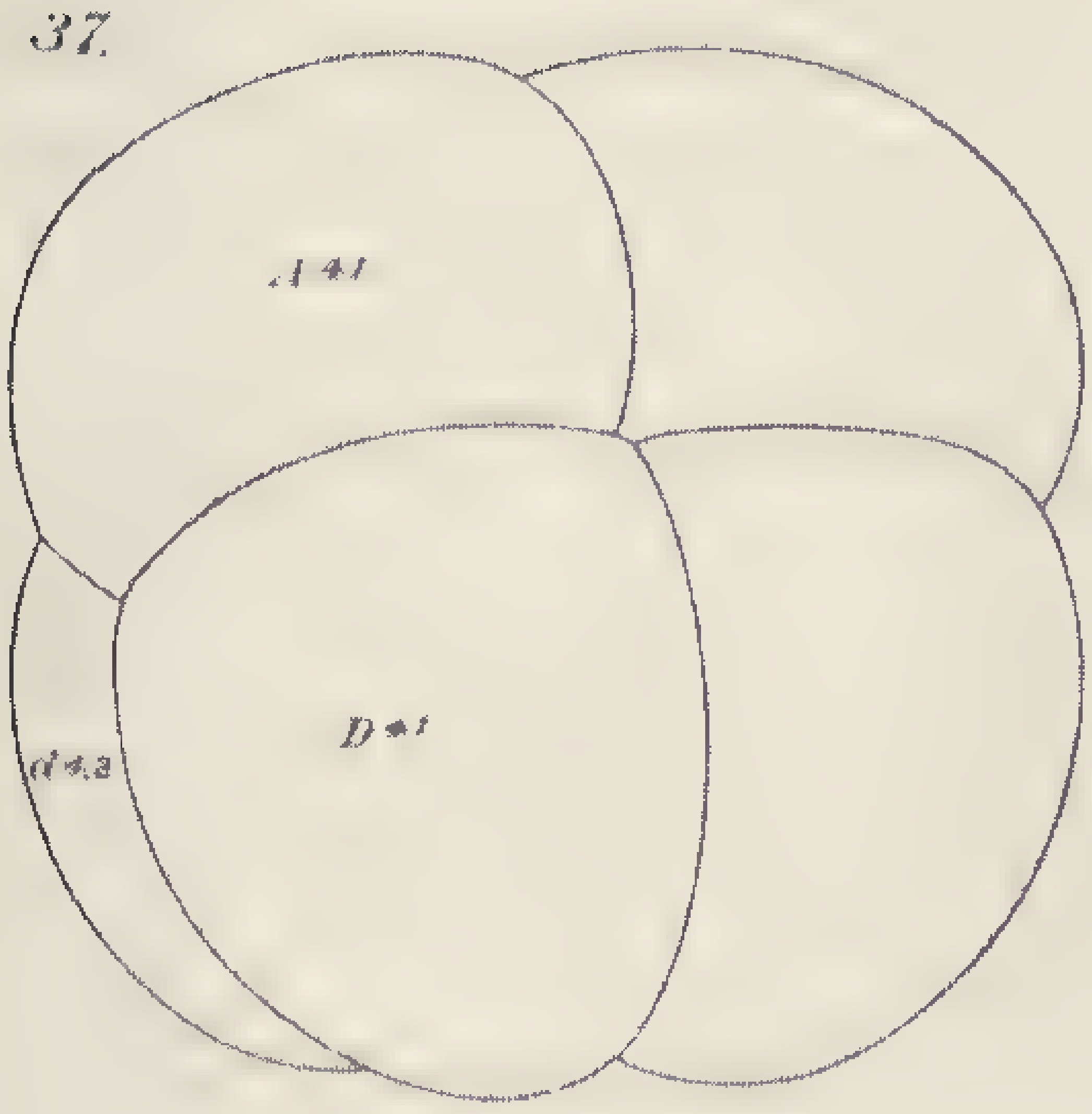

A4.1
a4.2
D4.1
d4.2

37.



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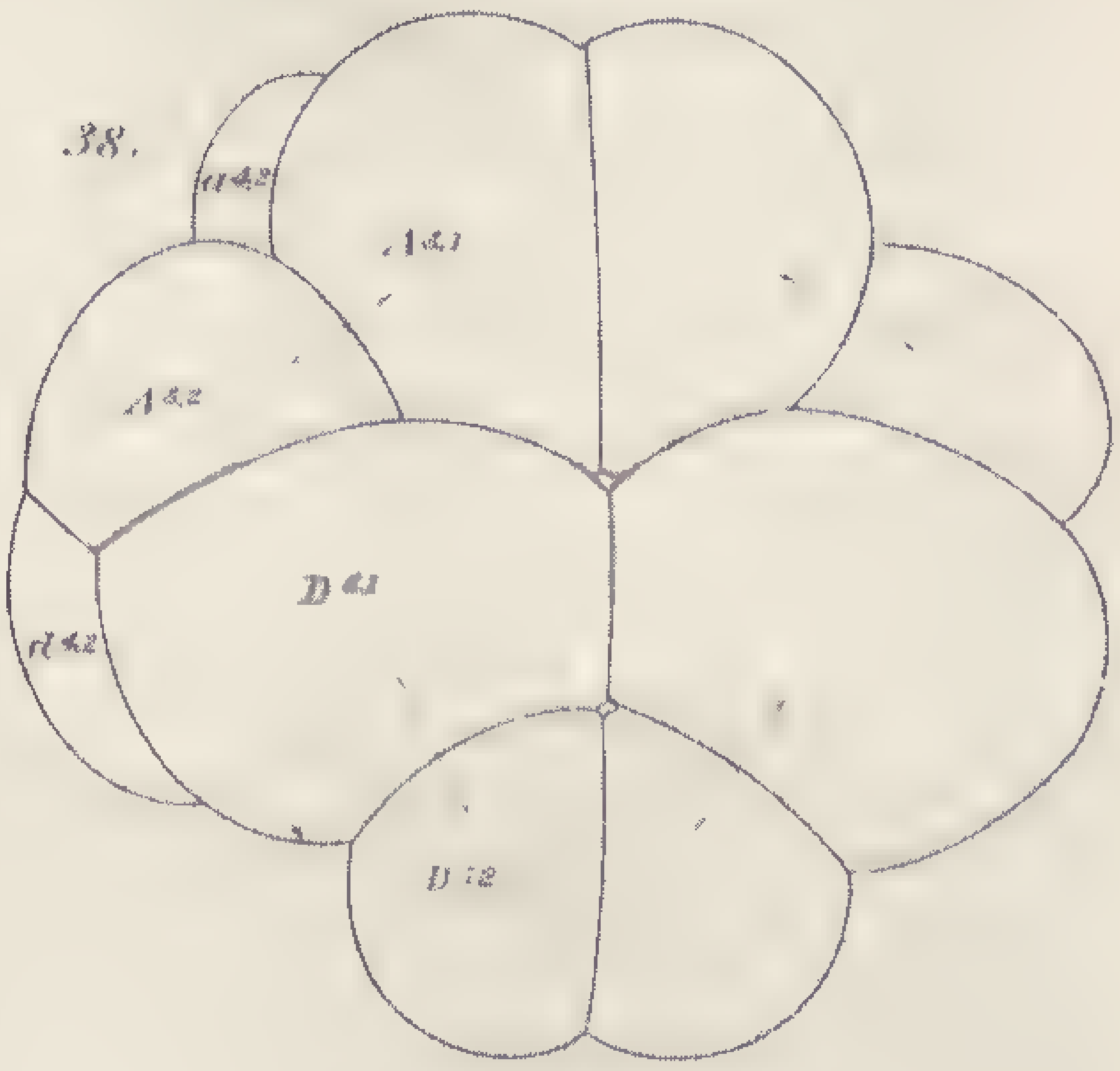

a4.2
A4.1
A4.2
D4.1
d4.2
D5.2

WEC del. B Meisel lith. Boston

PLATE VII.

Magnification of all Figures, except 43 and 44, 315 diameters; magnification of Figs. 43 and 44, about 300 diameters.

Figs. 39–42. Four later views of the egg shown in Figs. 35–38, Plate VI.; same (ventral) aspect.

Fig. 39. 16-cell stage, just formed. This view five minutes later than that in Fig. 38.

Fig. 40. 16-cell stage, five minutes later.

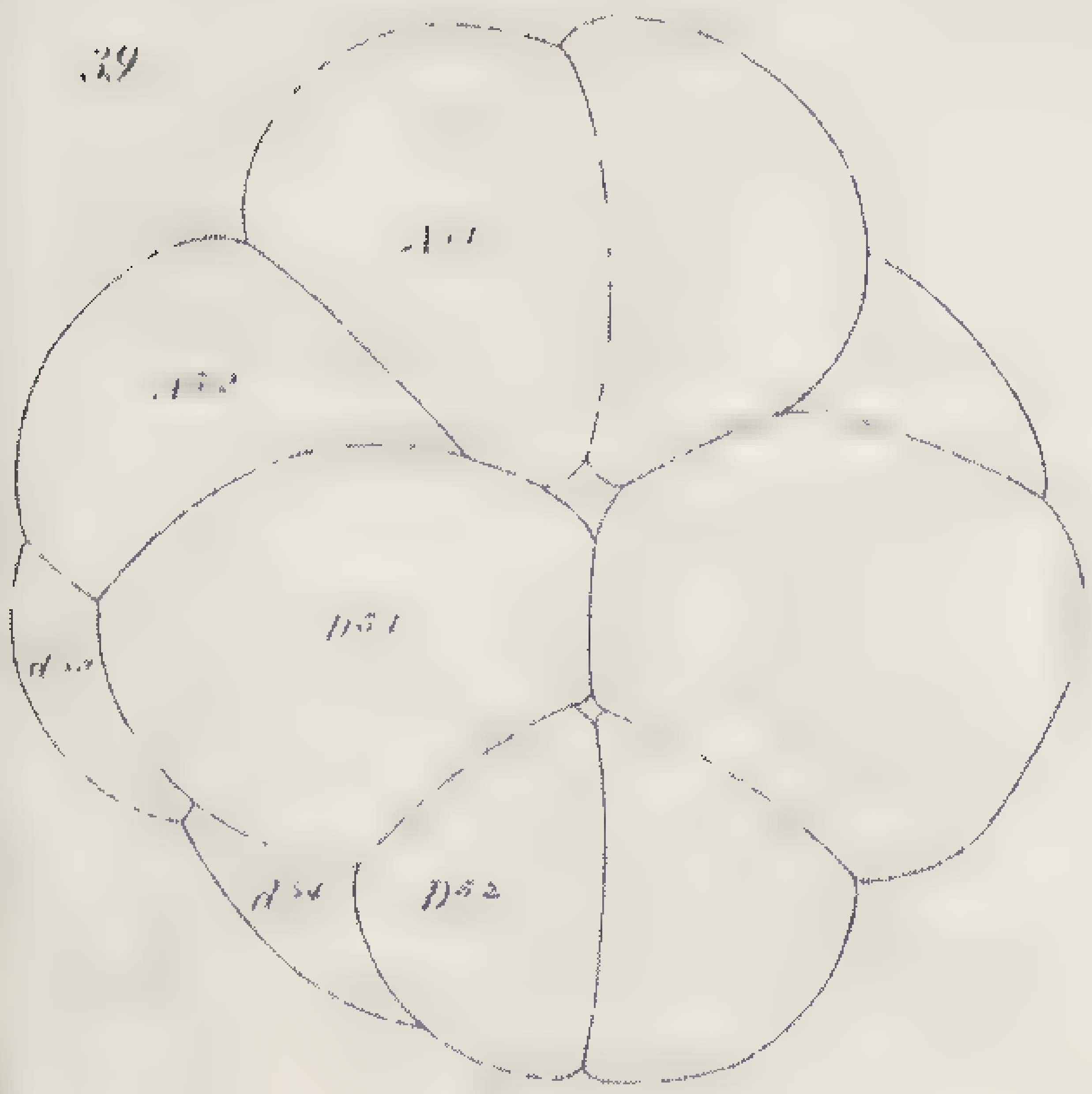
Fig. 41. 16-cell stage, ten minutes later still. Arrows indicate the direction of spindles.

Fig. 42. 24-cell stage, just formed, some minutes later than the last view.

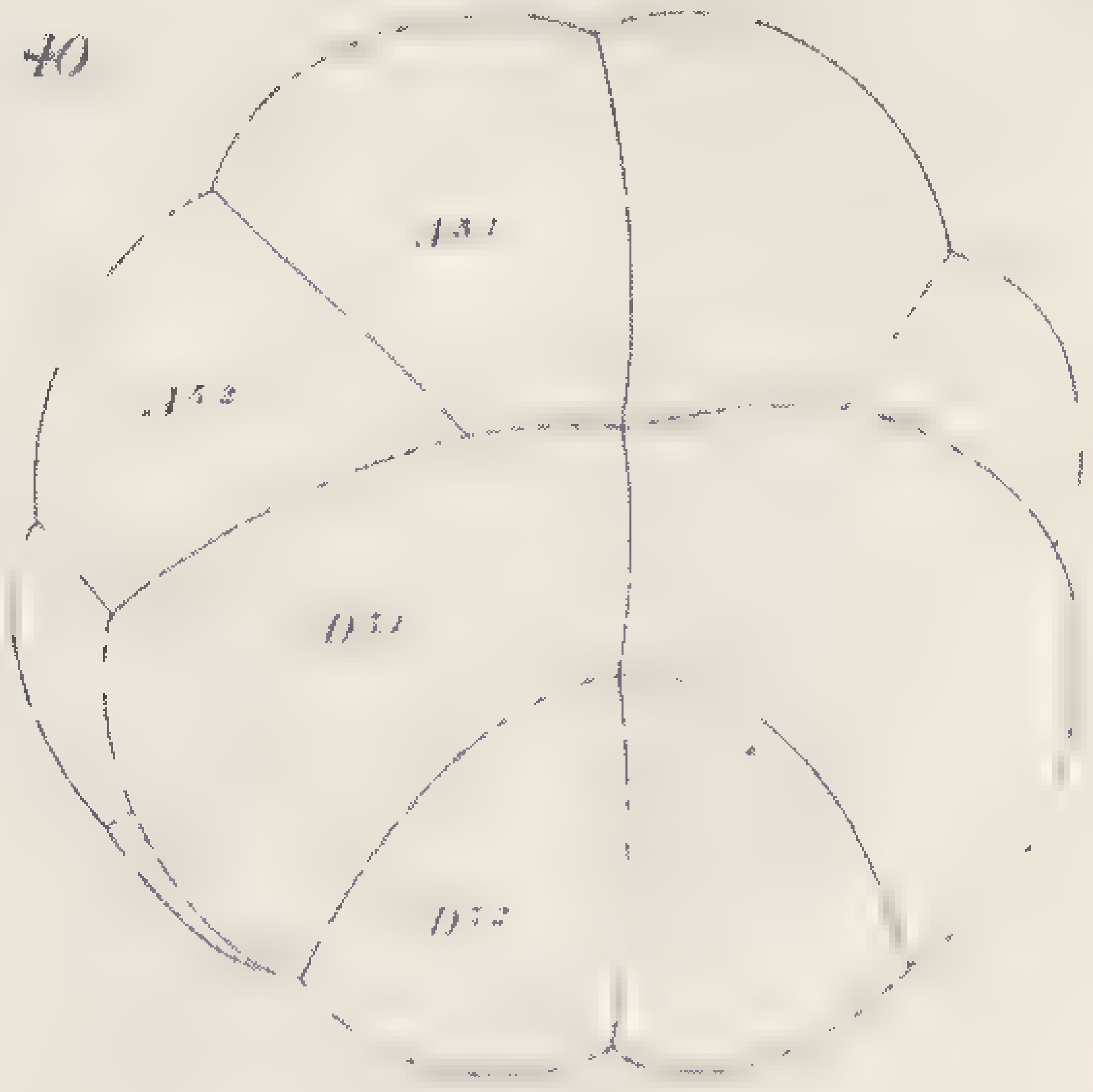
Fig. 43. A late 24-cell stage, viewed from the right side.

Fig. 44. Optical section of the same egg, near the median plane. The cells of the dorsal hemisphere are in mitosis.

39


A5.1
A5.2
d5.3
p5.1
d5.4
p5.2

40


A5.1
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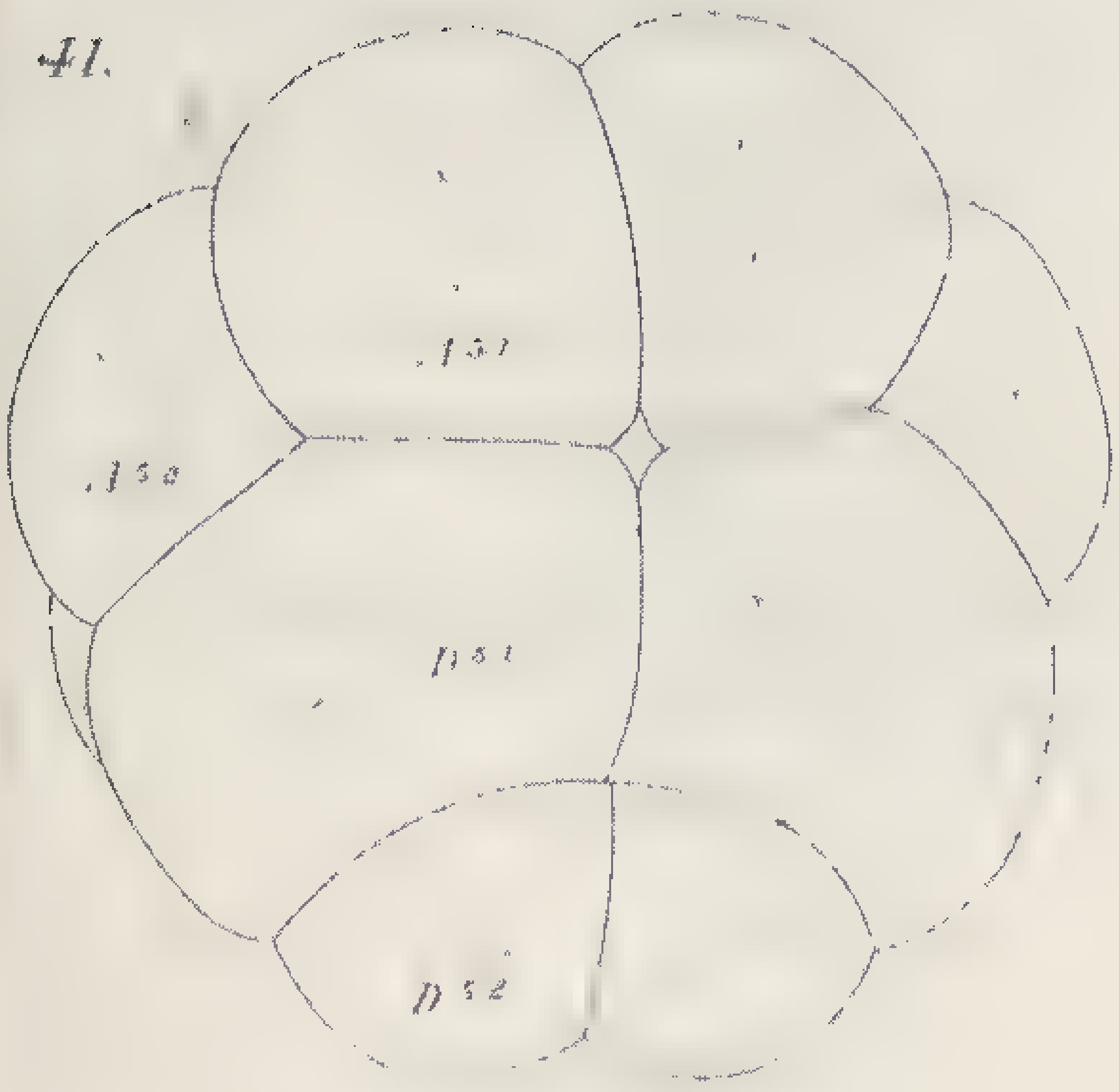
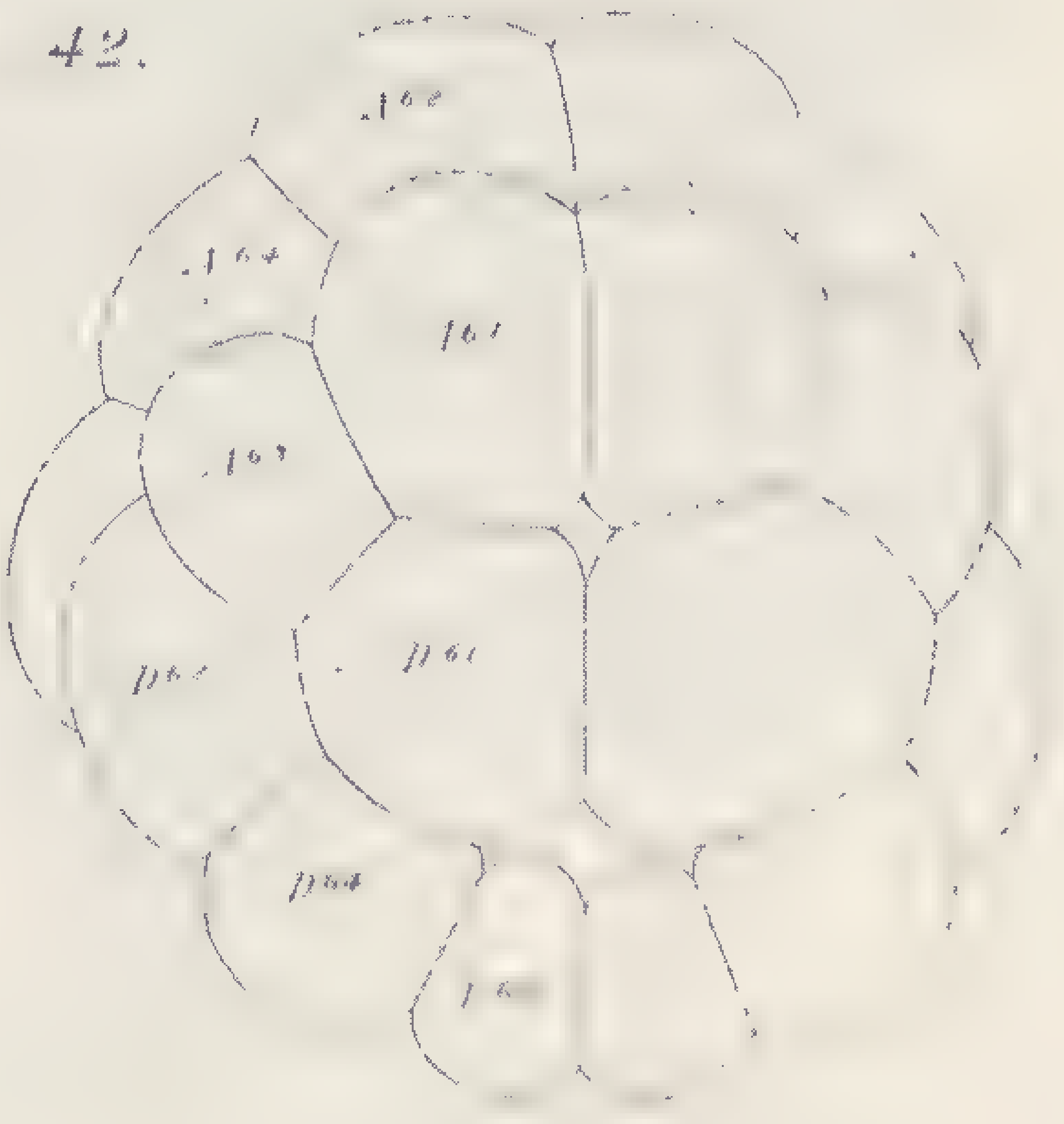
p5.1
p5.2

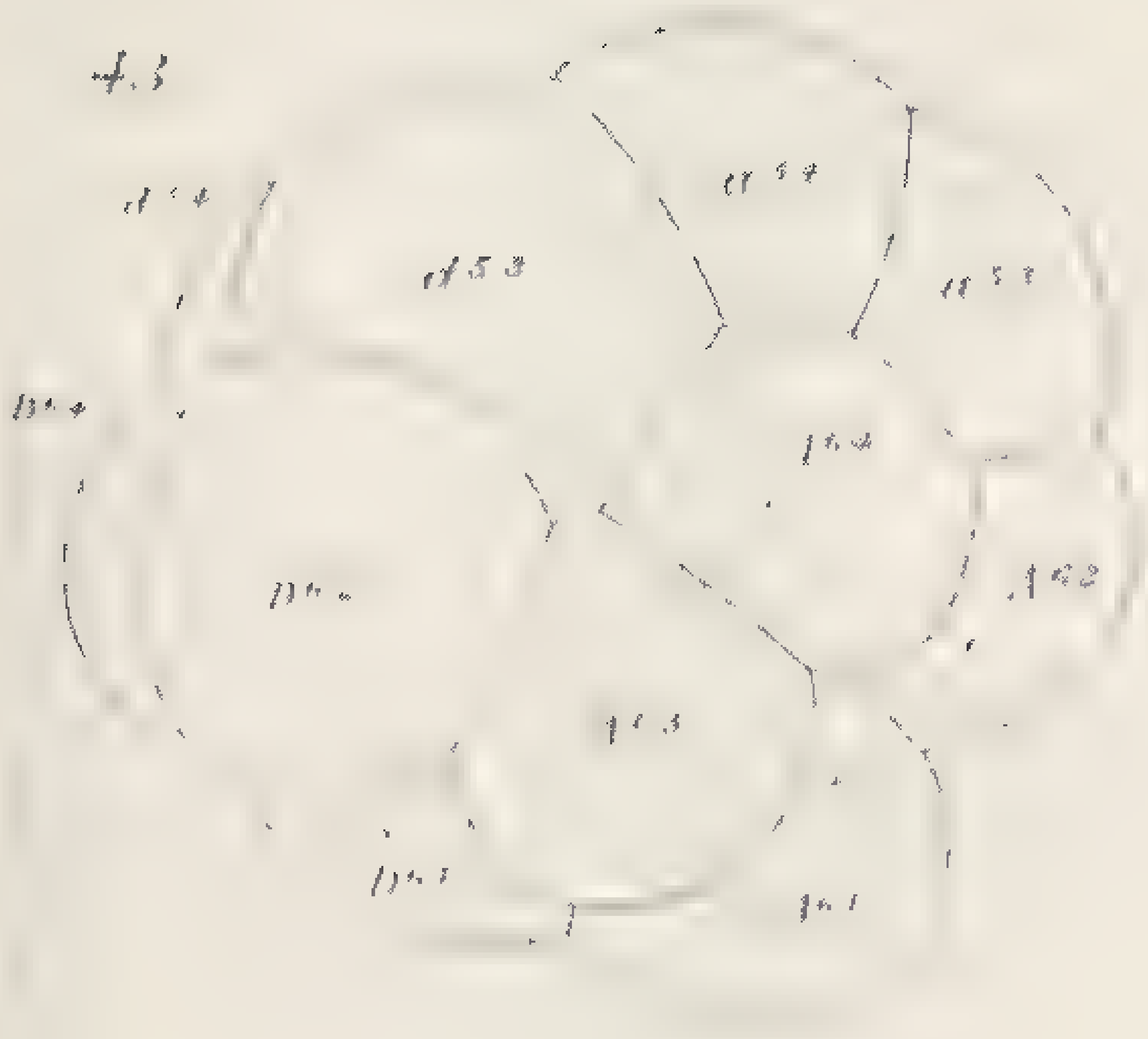
41.


A5.1
A5.2
p5.1
p5.2

42.


A6.2
A6.4
A6.1
A6.3
p6.2
p6.1
p6.4
p6.3

43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44

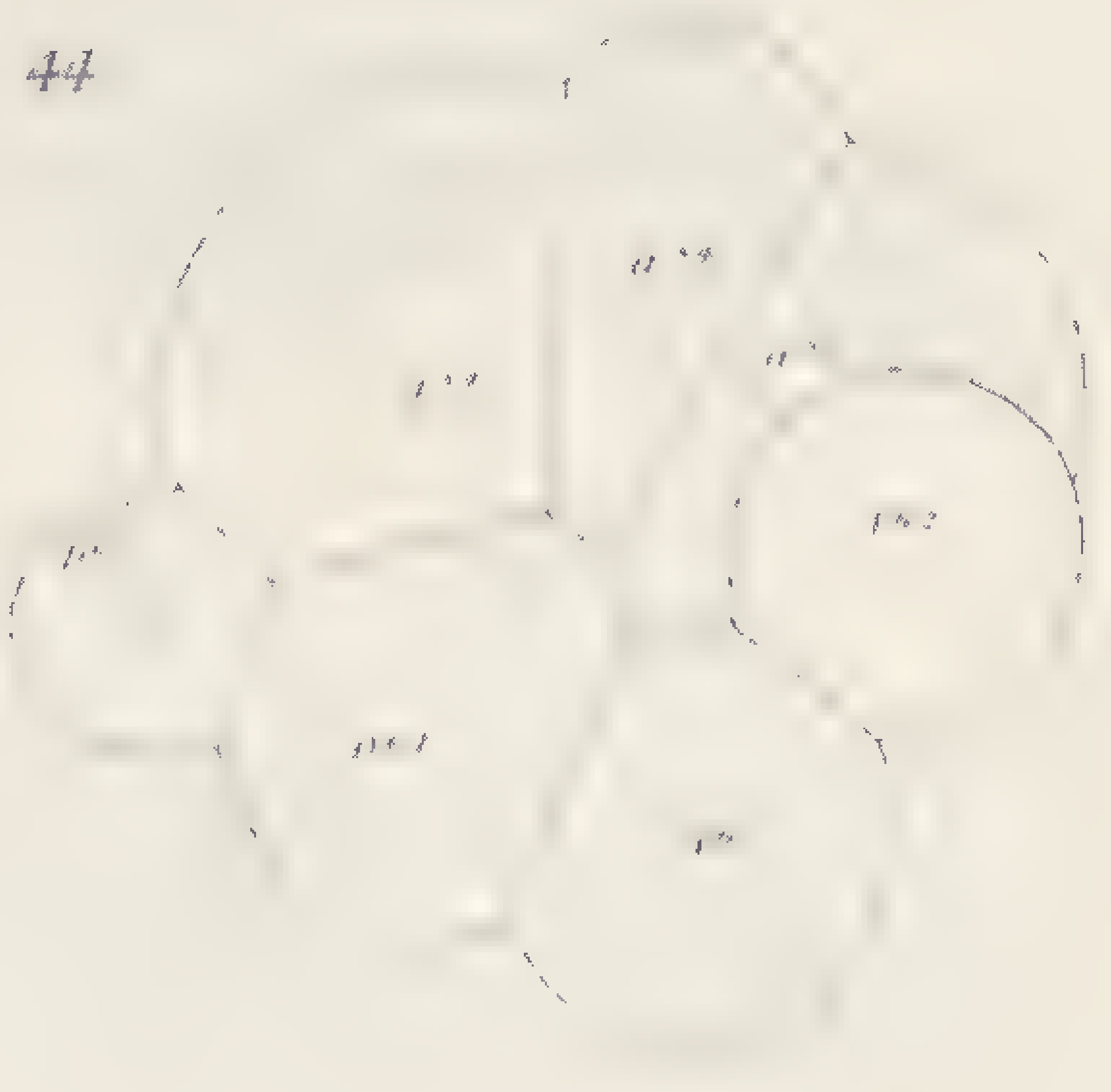


PLATE VIII.

Six successive views (obliquely from the left, above, and behind) of a living egg; magnification, 315 diameters. Arrows indicate the direction of spindles.

Fig. 45. 2-cell stage. *x*, region of finely granular protoplasm (cf. description of Plate III. Fig. 17).

Fig. 46. 4-cell stage, just formed.

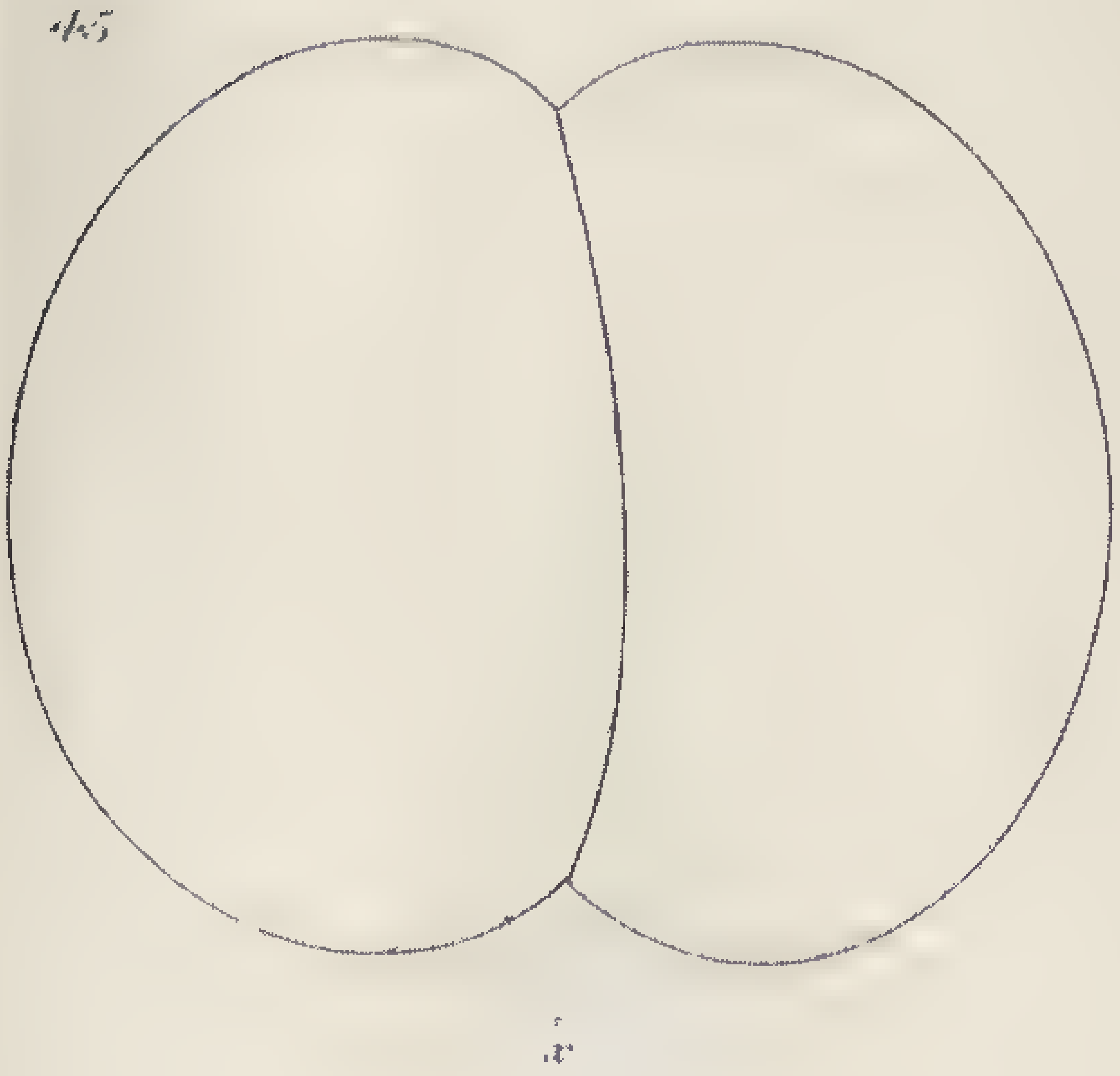
Fig. 47. 8-cell stage, approaching.

Fig. 48. 8-cell stage, fully formed (viewed as a transparent object).

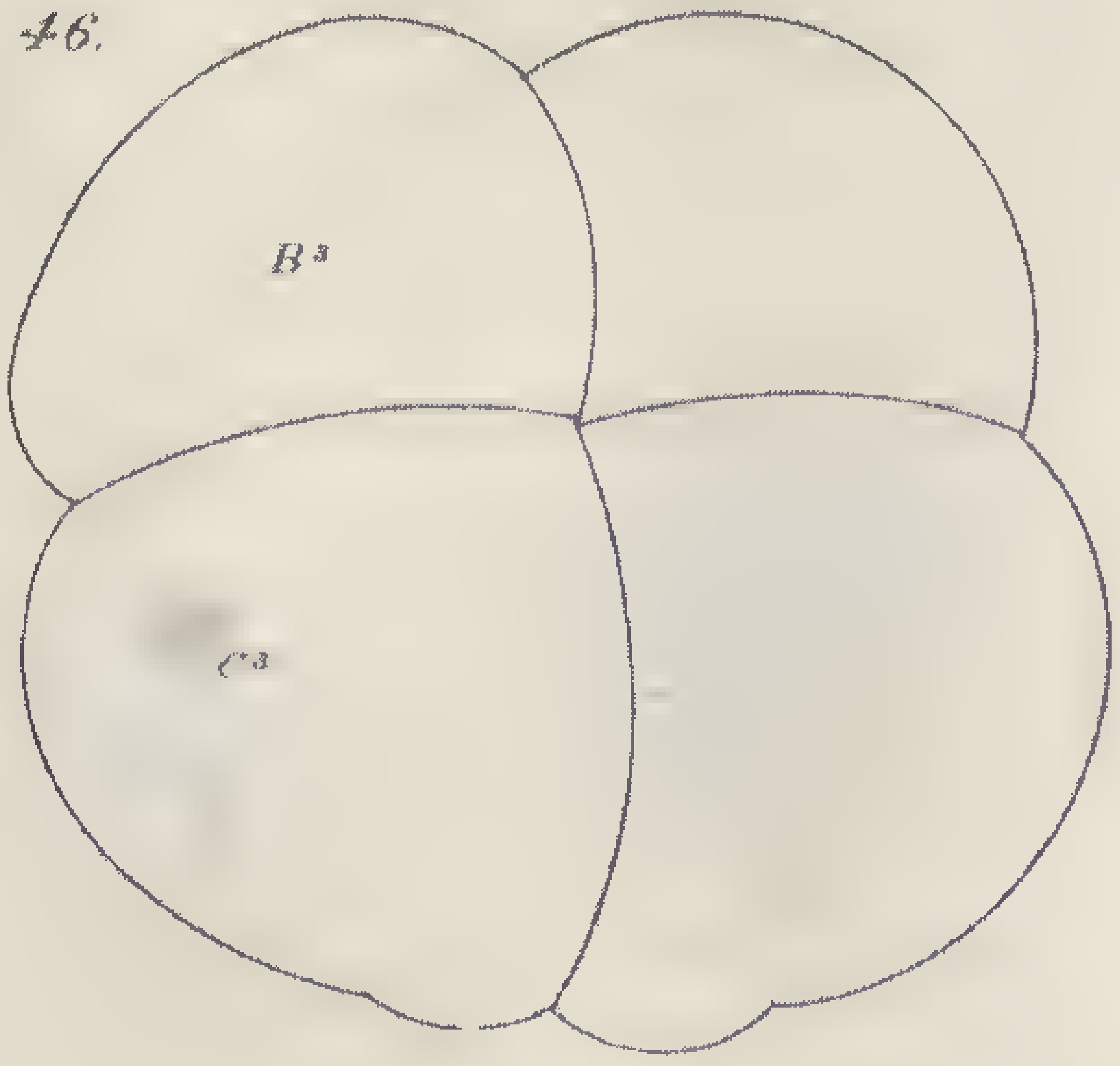
Fig. 49. 16-cell stage.

Fig. 50. 24-cell stage. During the formation of the 24-cell stage, the egg has rotated so that the view is almost exactly dorsal.

45.



46.



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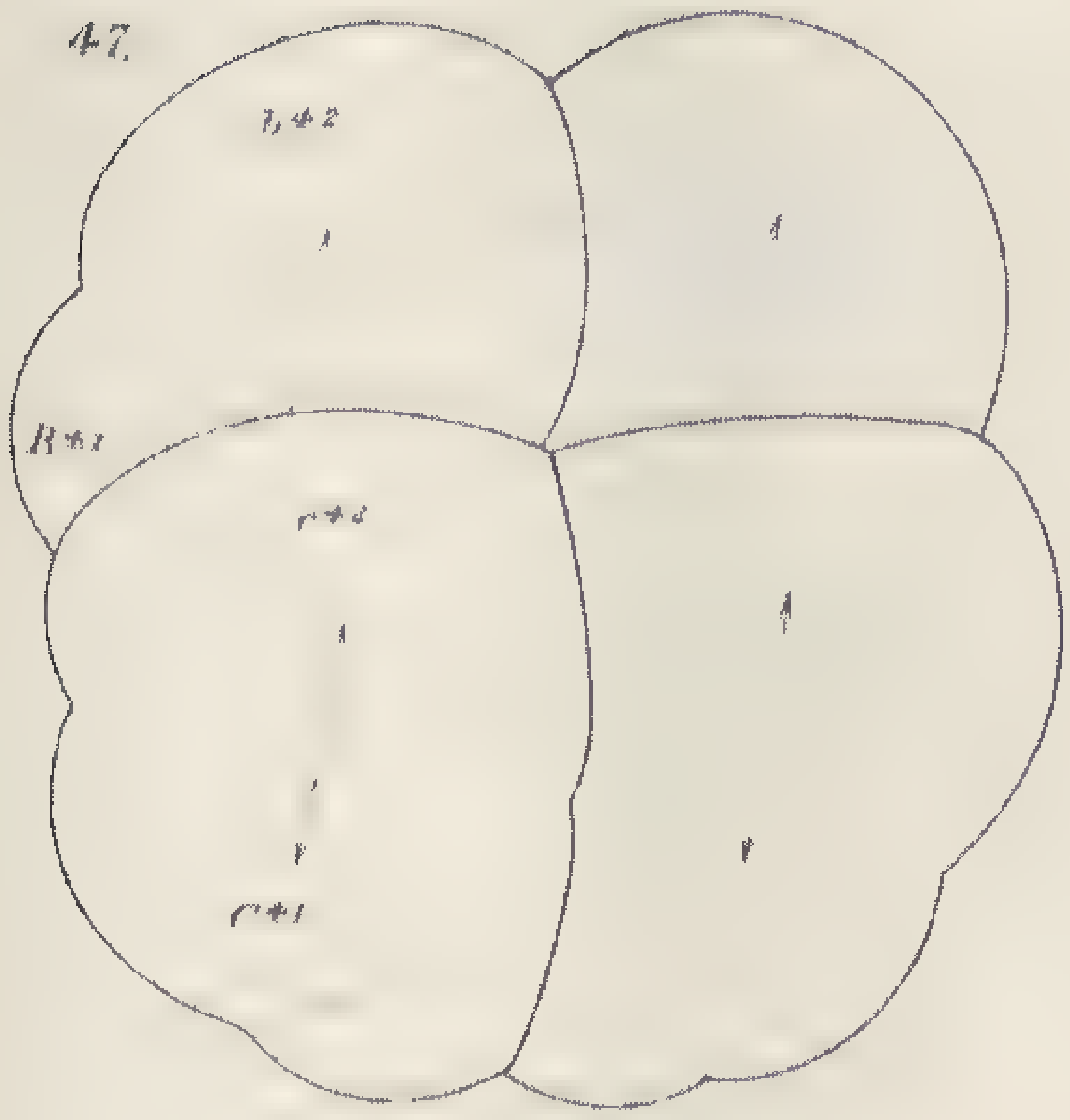
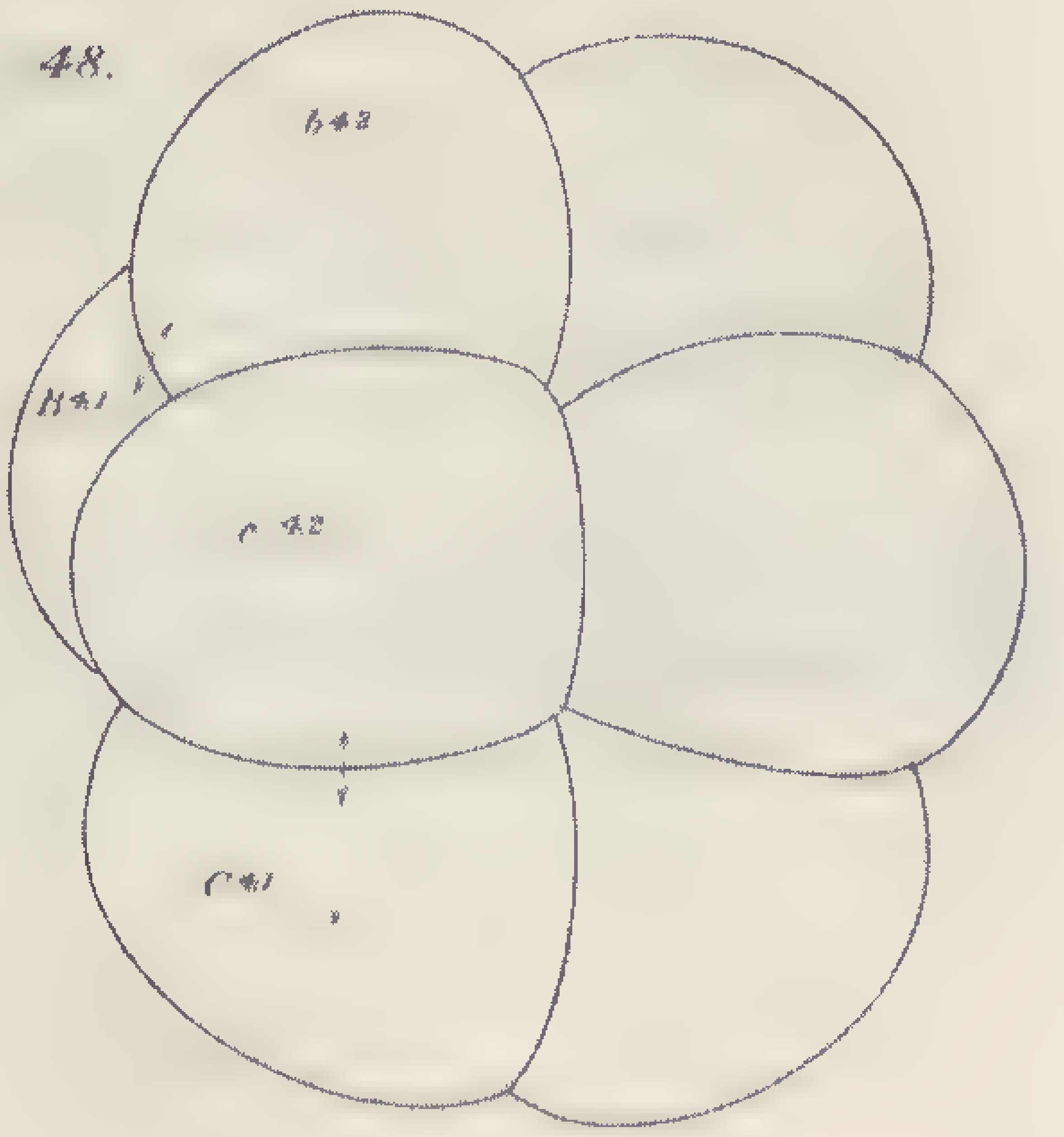

b^{42}
B^{41}
c^{42}
C^{41}

48.


b^{42}
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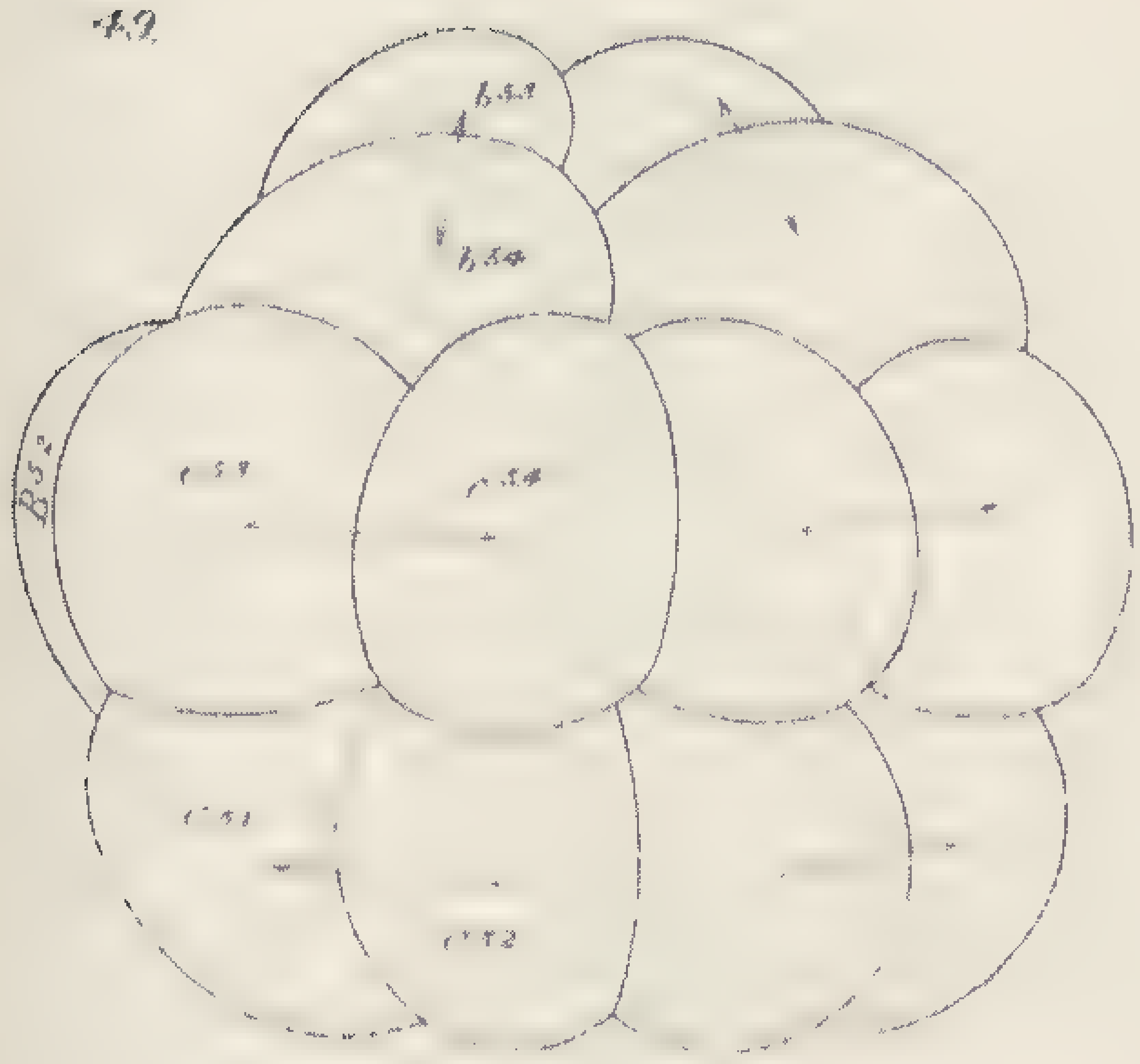
c^{42}
C^{41}

49.


b^{53}
b^{54}
B^{52}
c^{53}
c^{54}
c^{51}
c^{52}

50.

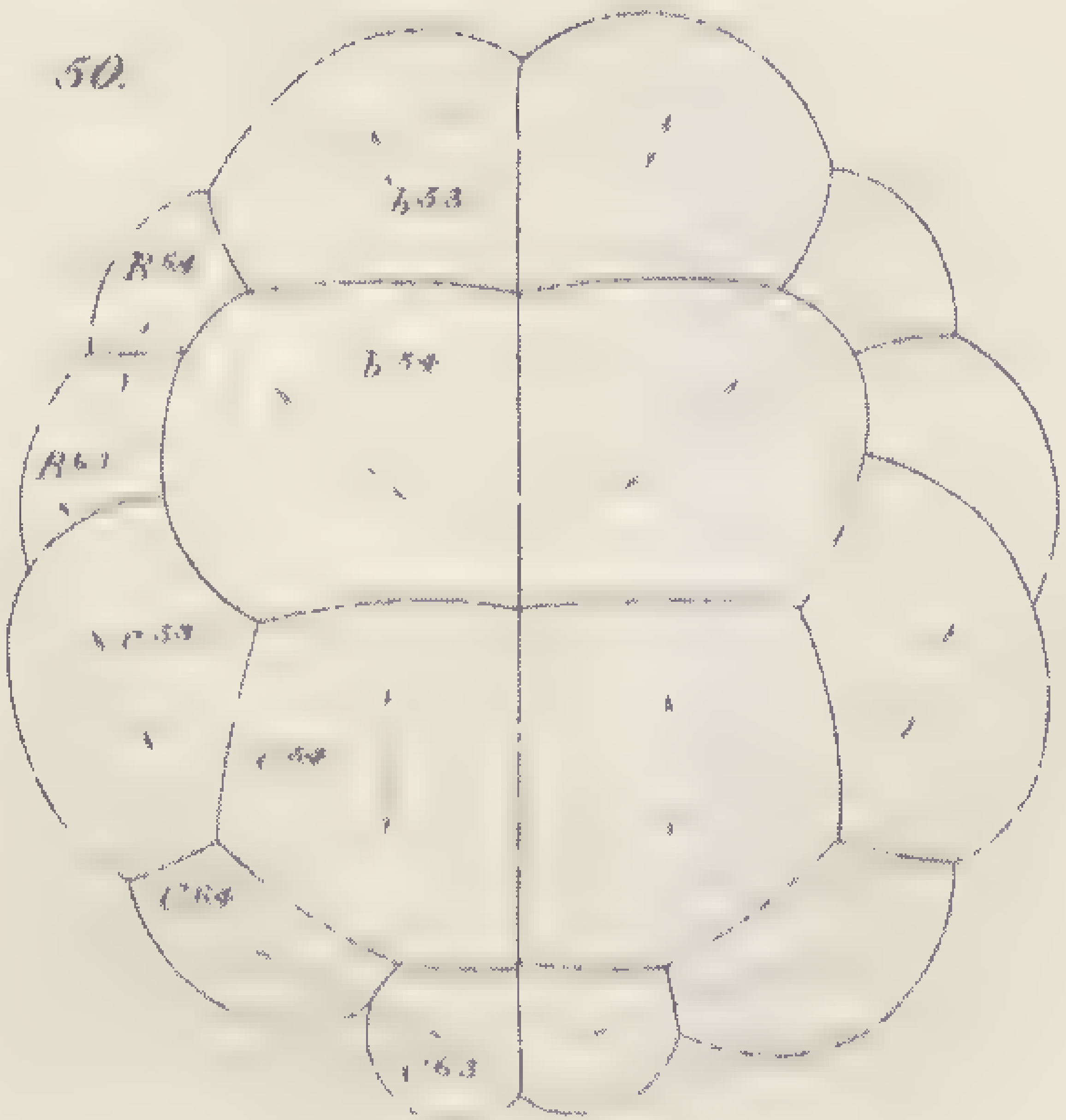

b^{53}
B^{54}
b^{54}
B^{63}
c^{53}
c^{54}
C^{64}
C^{63}

PLATE IX.

Surface views of preparations; magnification, 400 diameters.

Fig. 51. 24-cell stage, ventral view.

Fig. 52. The same egg, in the same stage, dorsal view (cf. Plate VII. Figs. 43 and 44).

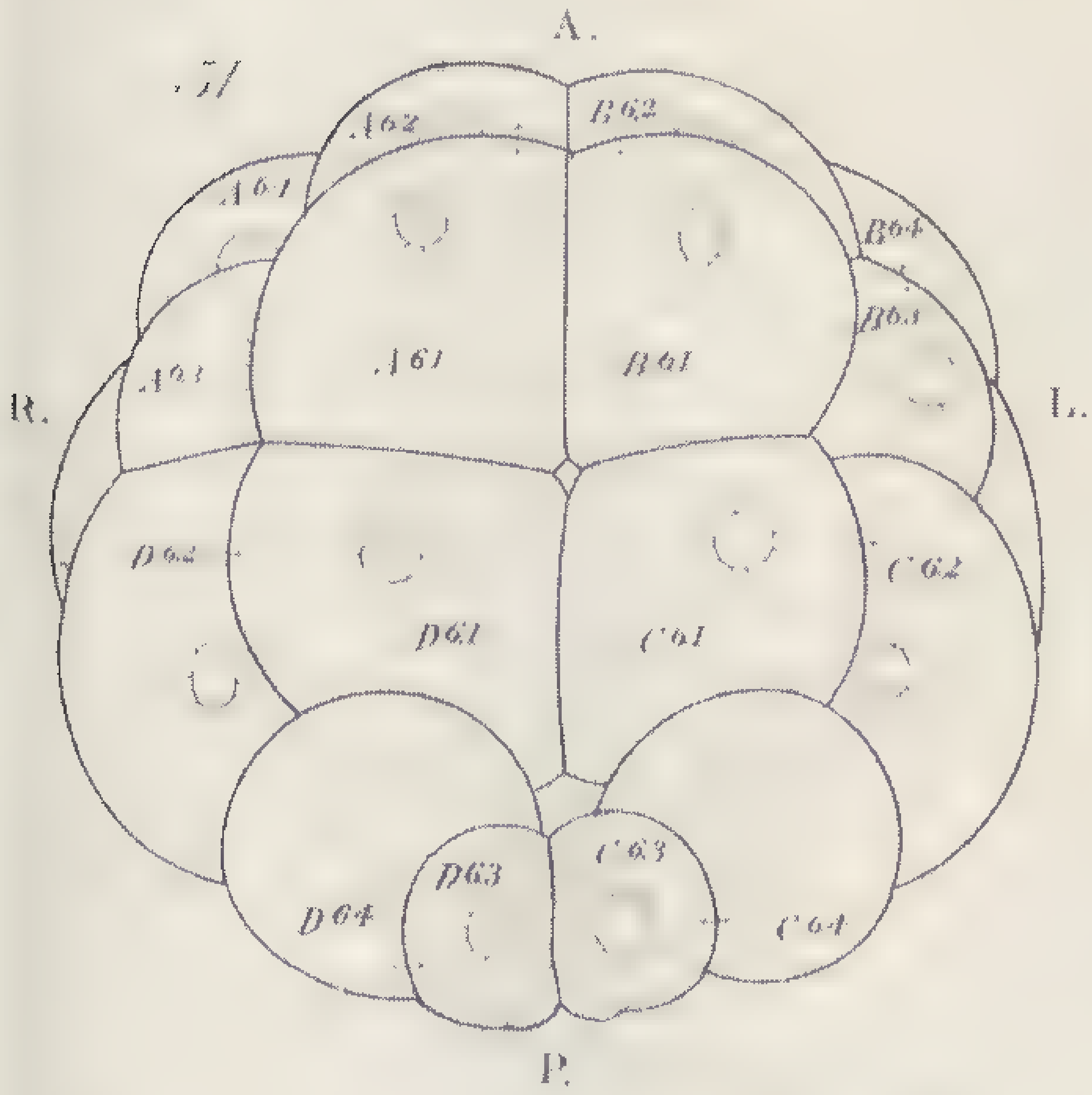
Fig. 53. 32-cell stage, ventral view.

Fig. 54. The same egg and stage, dorsal view.

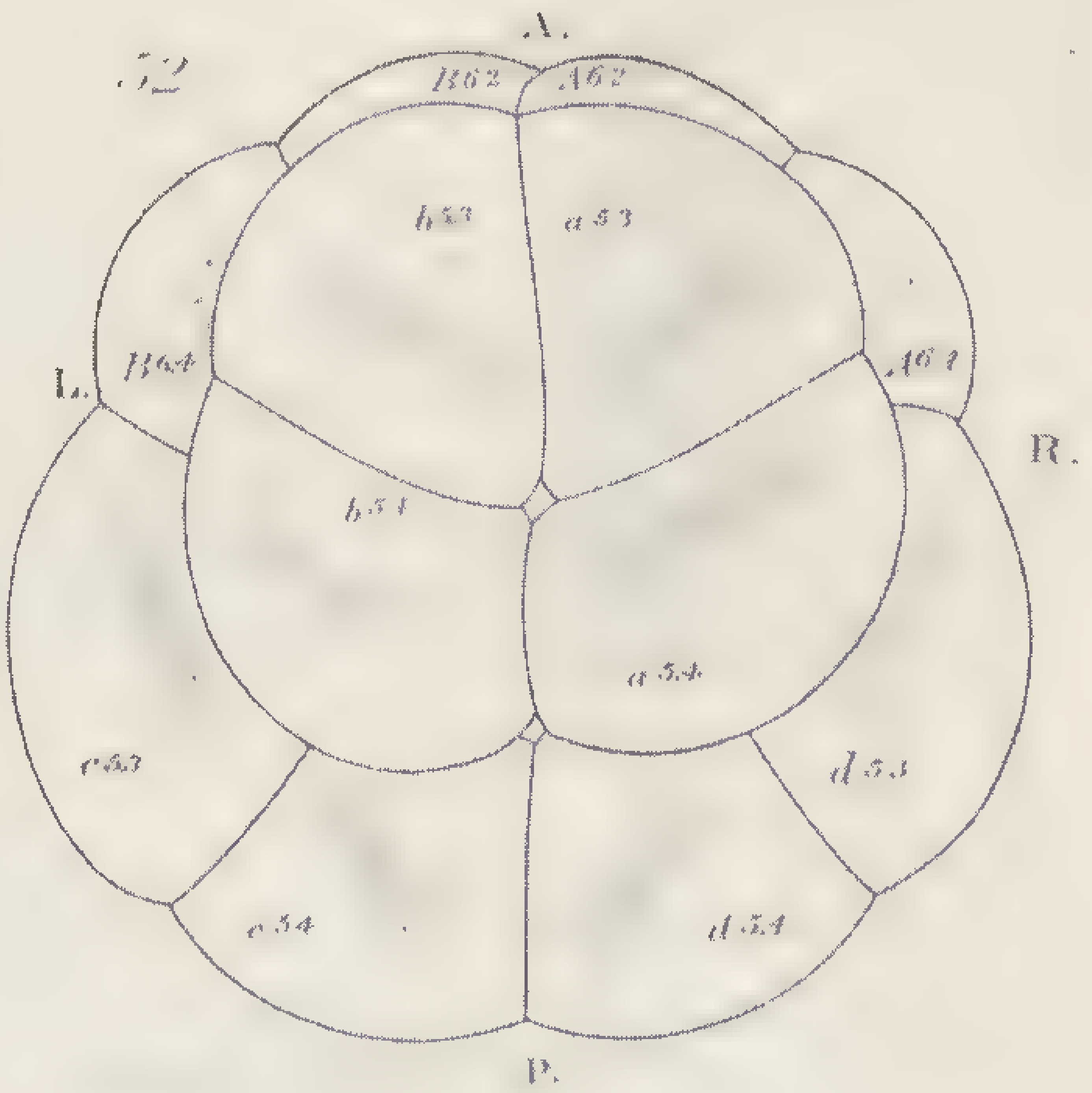
Fig. 55. 46-cell stage, ventral view.

Fig. 56. The same egg and stage, dorsal view.

51


A.
A62
B62
A64
B64
B63
A63
A61
B61
R.
L.
D62
D61
C61
C62
D64
D63
C63
C64
P.

52


A.
B62
A62
b53
a53
L.
B64
A64
R.
b54
a54
c53
d53
c54
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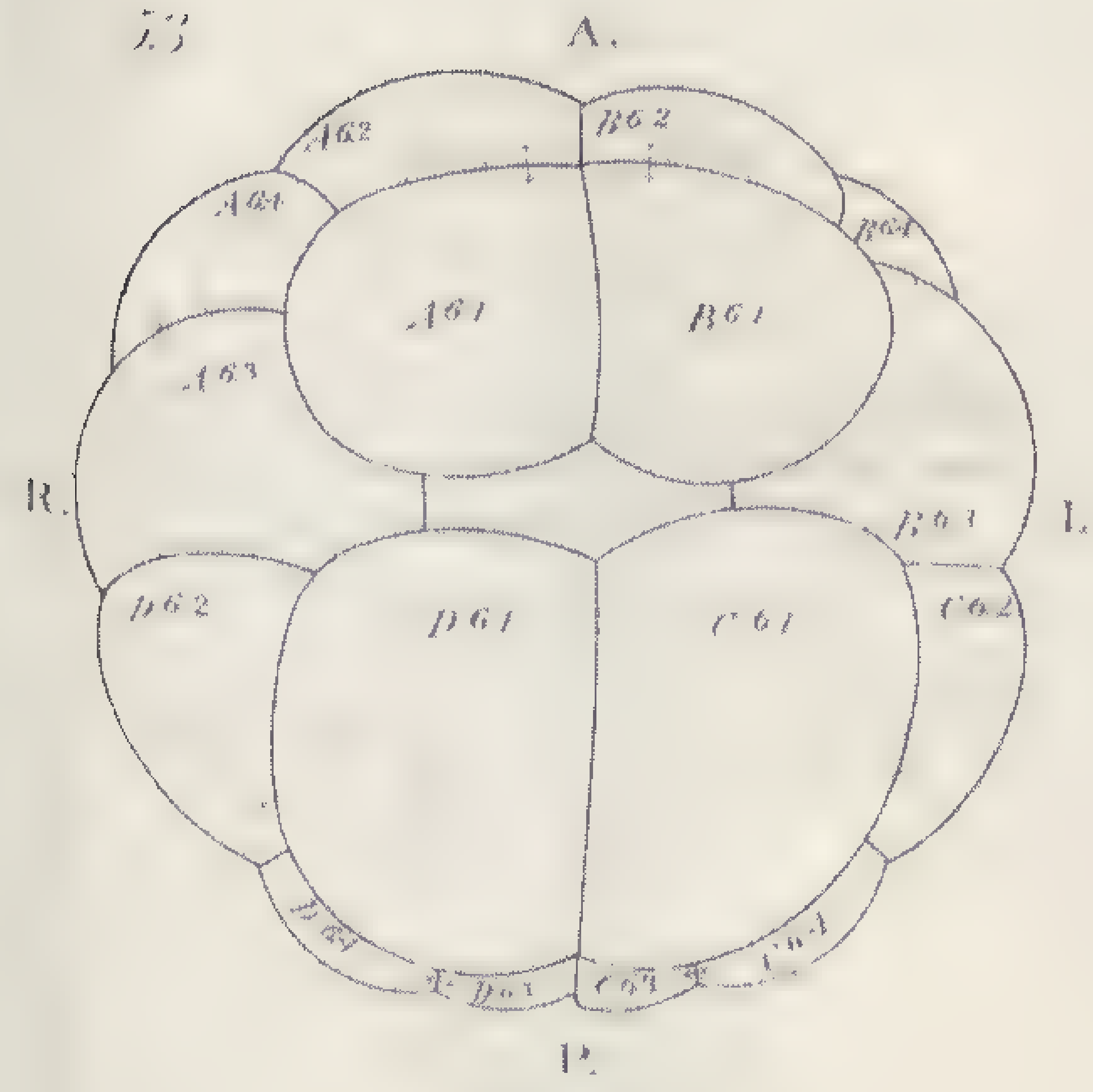
P.

53


A.
A62
B62
A64
B64
A61
B61
A63
R.
B63
L.
D62
D61
C61
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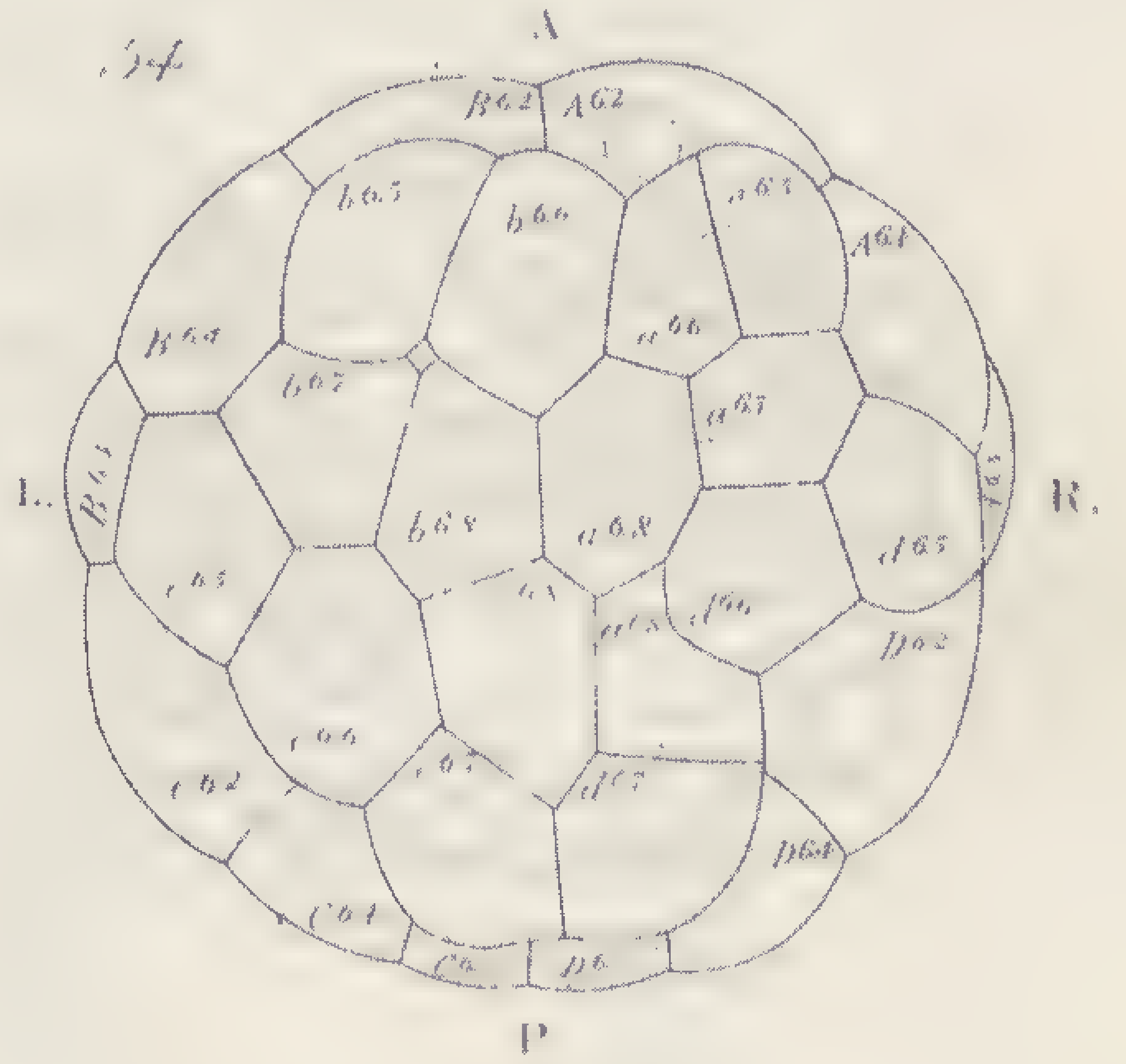
D64
D63
C63
C64
P.

54


A
B62
A62
b65
b66
a65
A64
B64
a66
b67
a67
L.
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b68
a68
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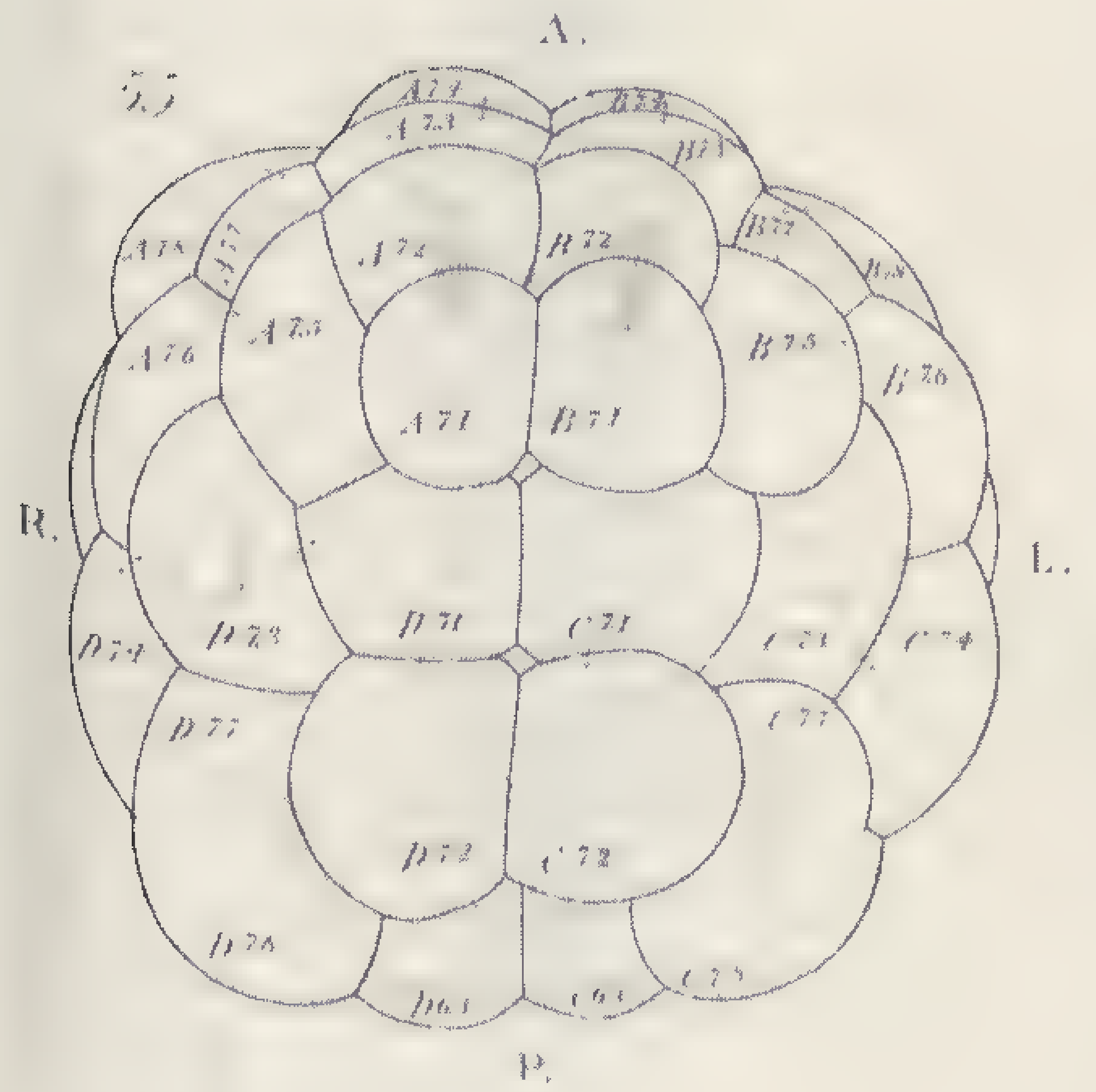
C64
C6
D6
P

55


A.
A74
B74
A73
B73
A78
A77
B77
B78
A72
B72
A76
A75
B75
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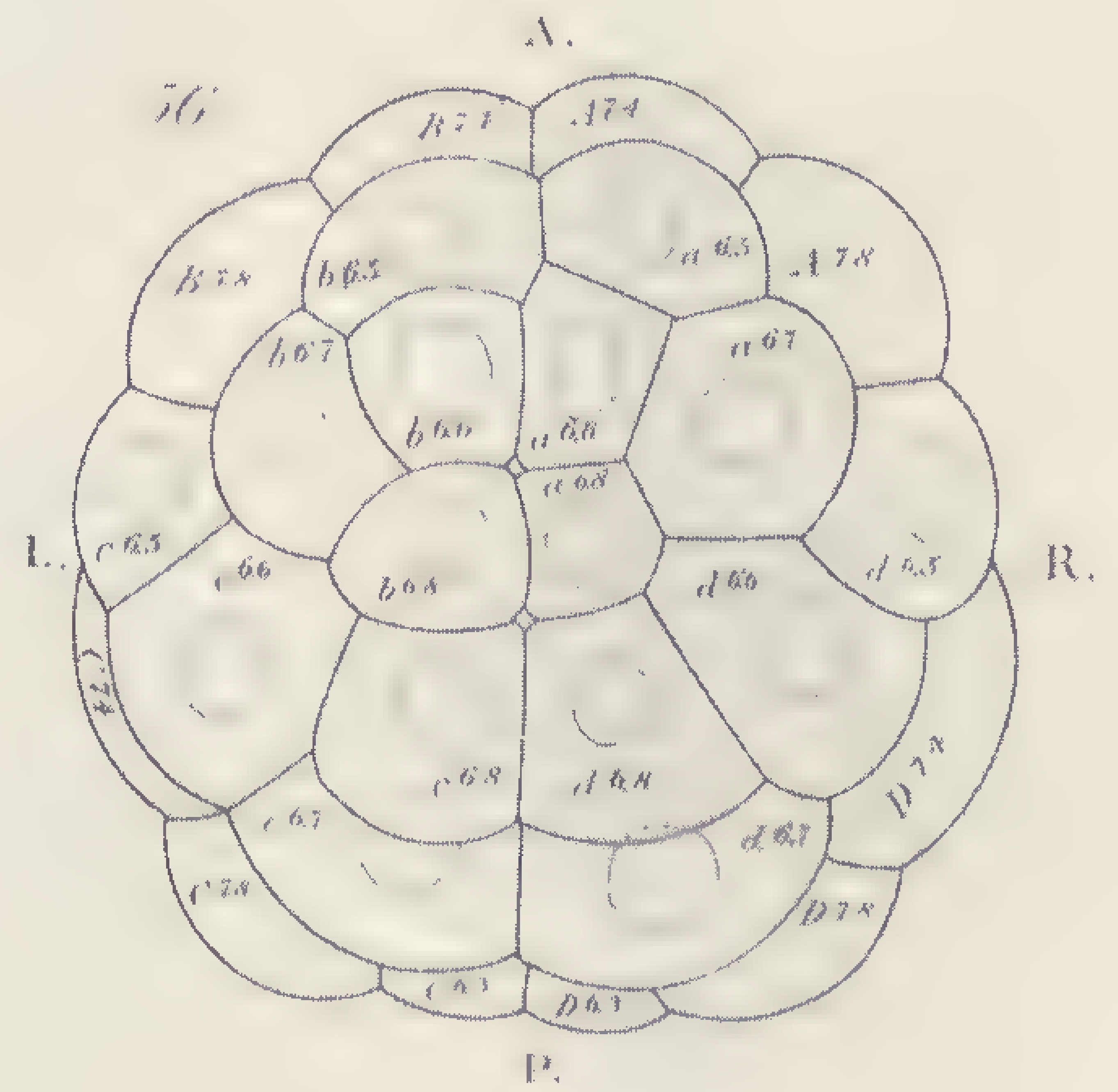
B71
R.
L.
D74
D73
D71
C71
C73
C74
D77
C77
D72
C72
D76
D63
C63
C75
P.

56


A.
B74
A74
B78
b65
a65
A78
b67
a67
b66
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L.
c65
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b68
d66
d65
R.
C74
c68
d68
D74
c67
d67
C78
D78
C63
D63
P.

PLATE X.

Figs. 57–62. Surface views of preparations; magnification, 560 diameters.

Fig. 57. 48-cell stage, viewed from behind.

Fig. 58. The same egg and stage, viewed from in front.

Fig. 59. 64-cell stage, ventral view.

Fig. 60. The same egg and stage, dorsal view.

Fig. 61. 76-cell stage, ventral view.

Fig. 62. The same egg and stage, dorsal view.

Note. — Consult general statement under Explanation of Plates. The cell $D^{8.6}$ and its mate in the left half of the Figure were stippled by mistake.

Figs. 63–70. Eight cross-sections from a series through an embryo in late 76-cell stage; magnification, 560 diameters. For position of sections in embryo, see horizontal lines 63–70 in Fig. 62.

Note. — In Fig. 63, the cells $D^{7.6}$ and $D^{8.14}$ should be stippled like their mates. In Fig. 67, the cell $C^{7.11}$ should be $c^{7.11}$.

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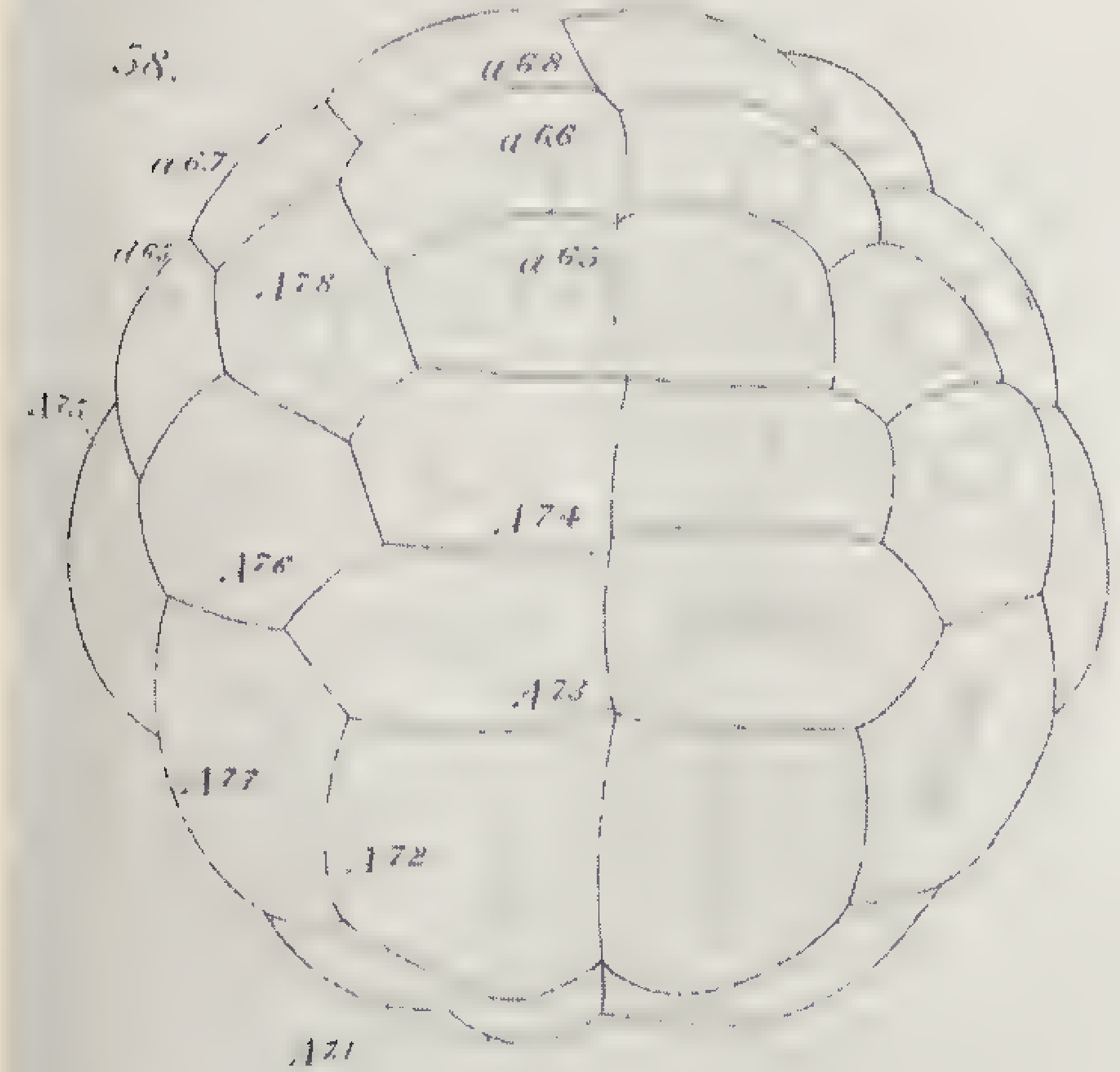
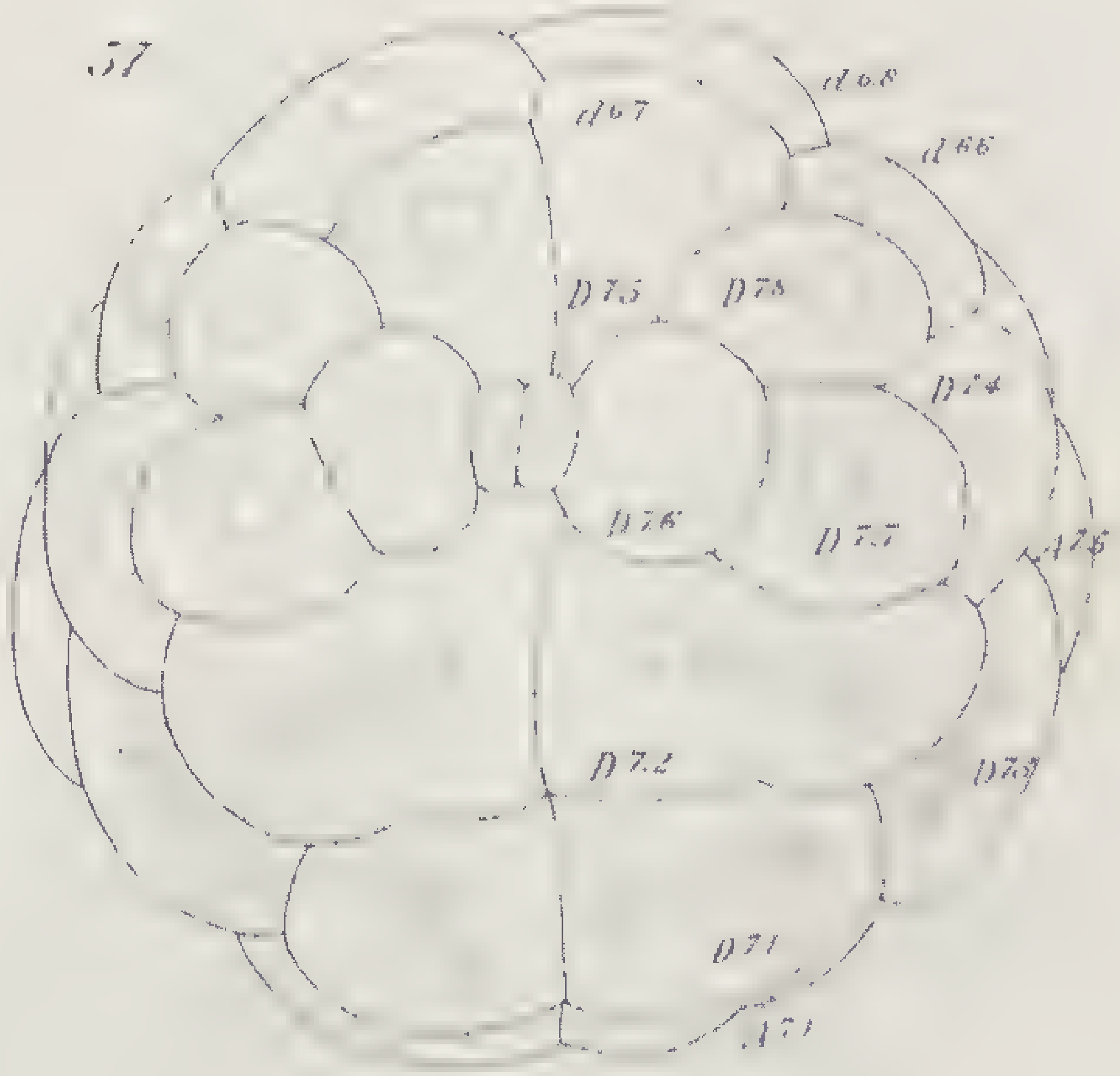
58.



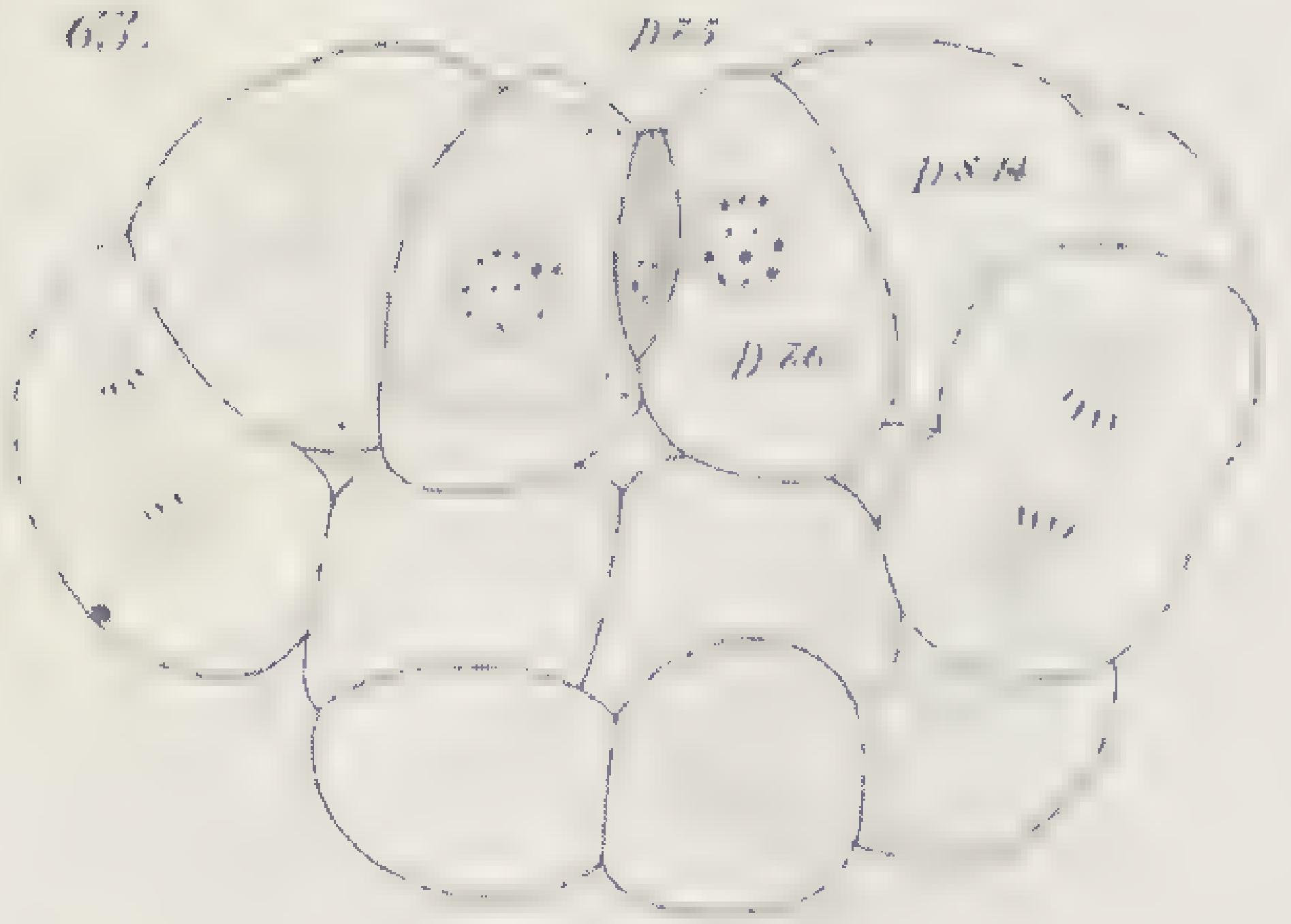
a6.8
a6.6
a6.7
a6.5
d6.5
A7.8
A7.5
A7.4
A7.6
A7.3
A7.7
A7.2
A7.1

57



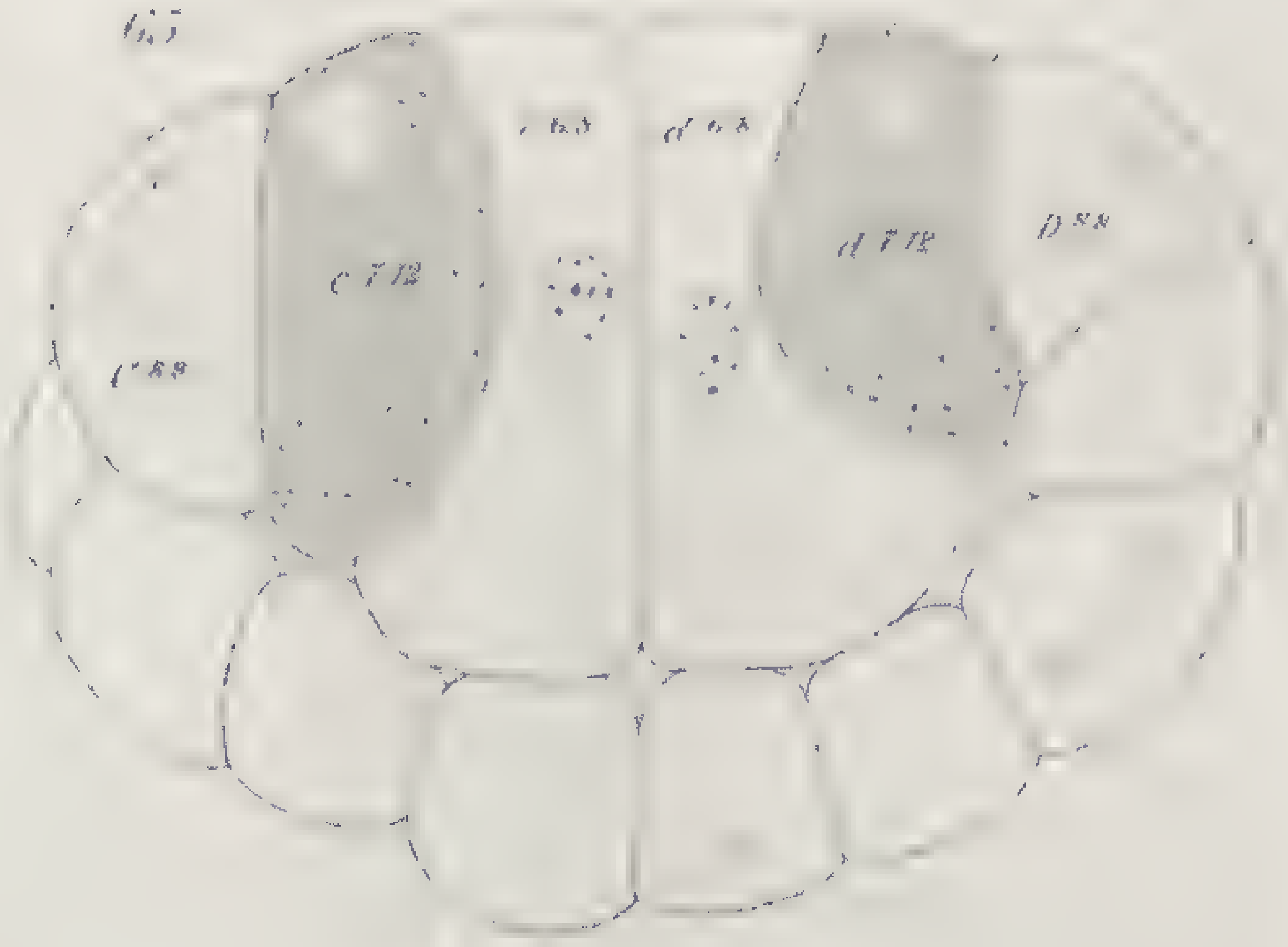
d6.7
d6.8
d6.6
D7.5
D7.8
D7.4
D7.6
D7.5
A7.6
D7.2
D7.3
D7.1
A7.1

63.



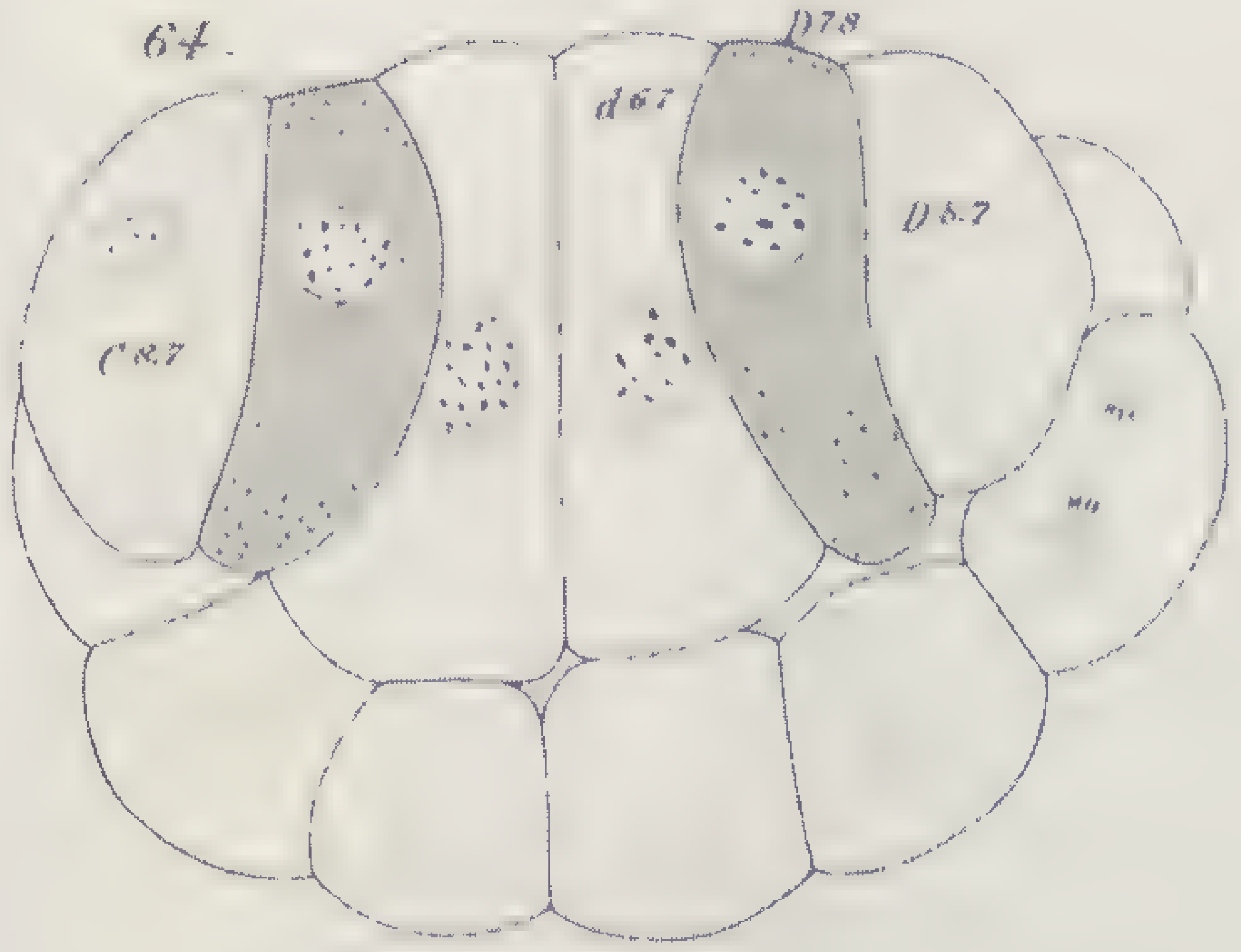
D7.5
D8.14
D7.6

65



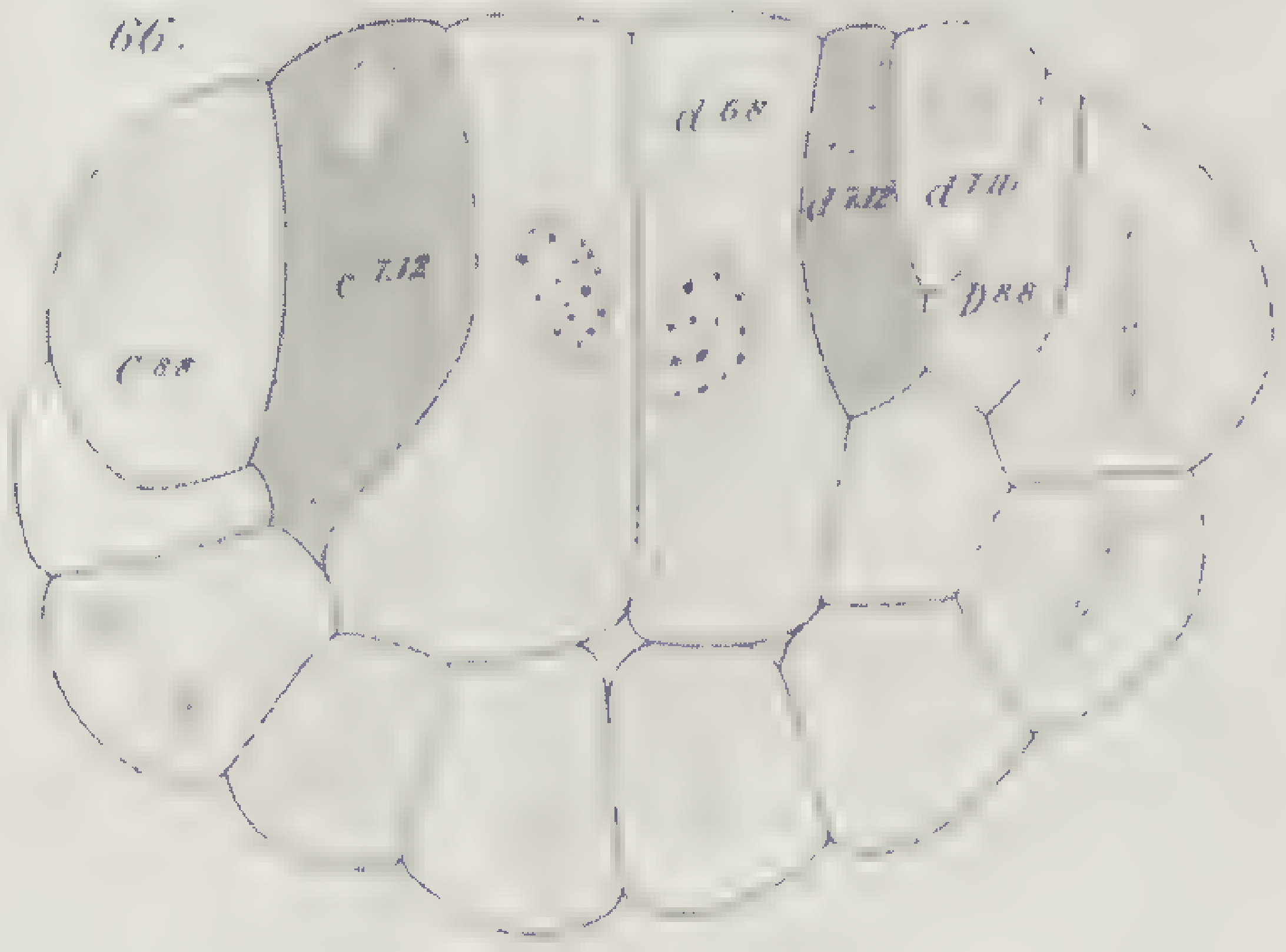
c6.5
d6.8
C7.12
d7.12
D8.8
C8.8

64.



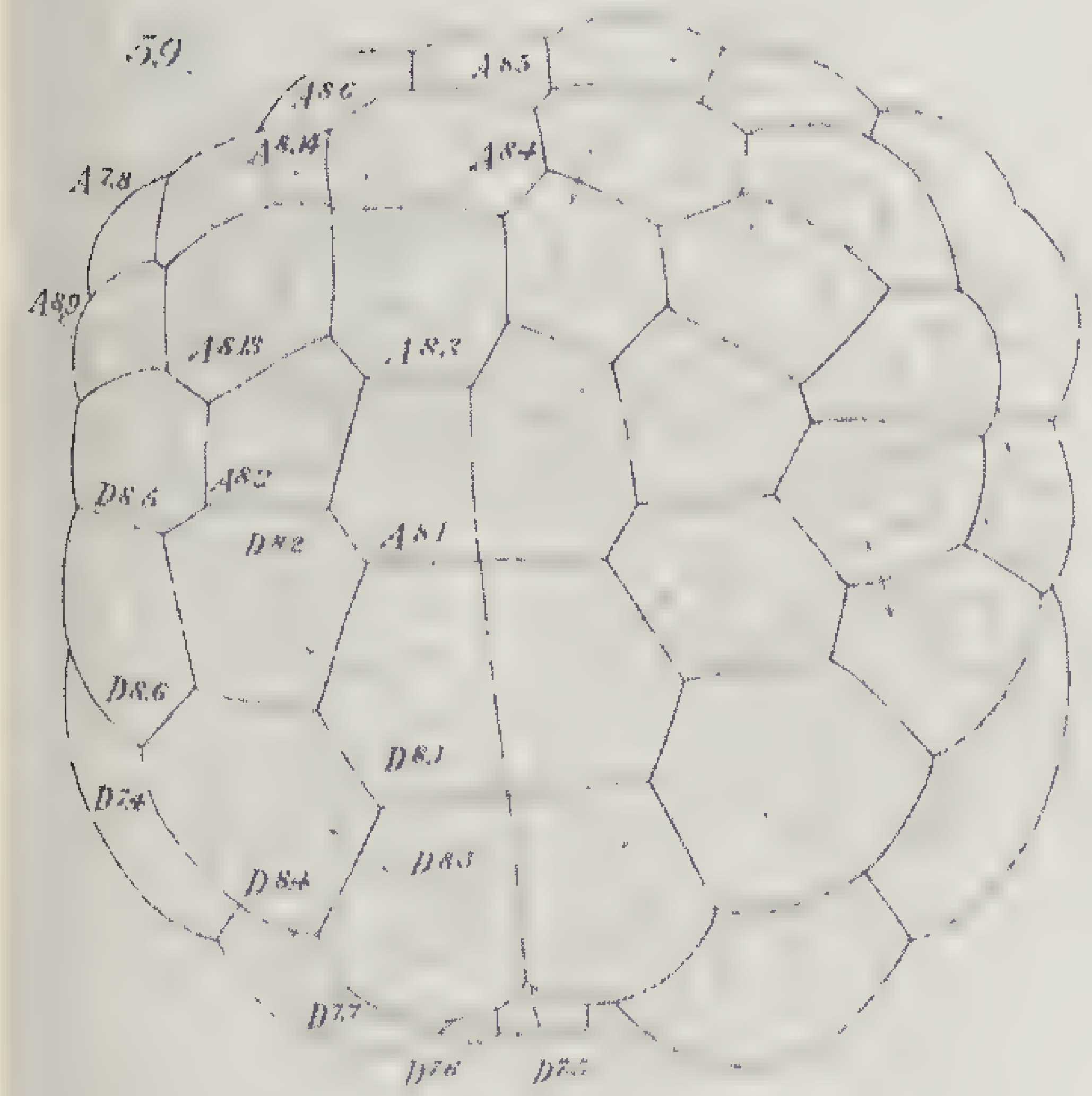
D7.8
d6.7
D8.7
C8.7

66.



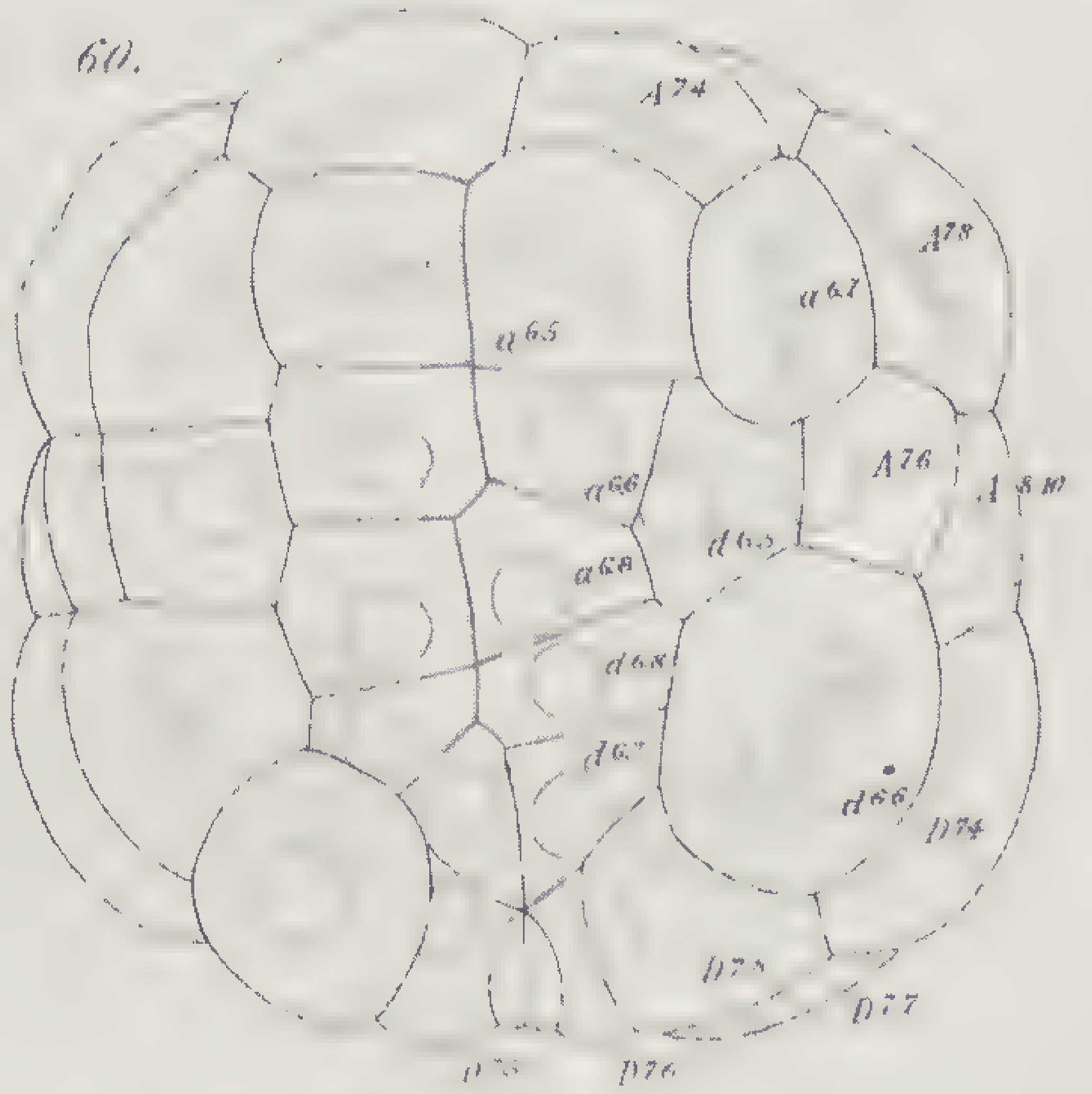
d6.8
d7.12
d7.16
C7.12
C8.8
D8.8

59.



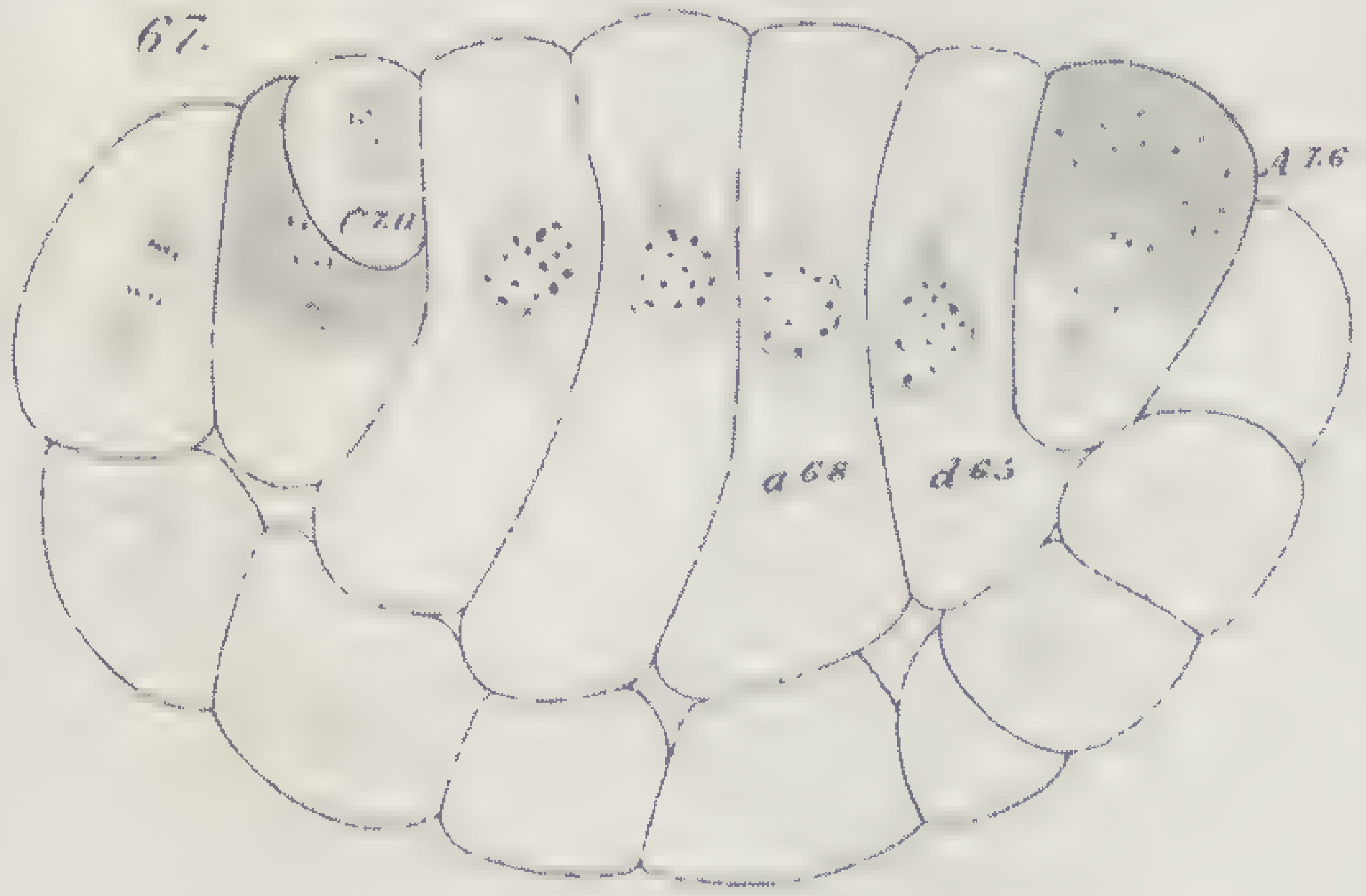
A8.6
A8.5
A8.14
A8.4
A7.8
A8.9
A8.13
A8.3
A8.2
D8.5
D8.2
A8.1
D8.6
D8.1
D7.4
D8.4
D8.3
D7.7
D7.6
D7.5

60.



A7.4
A7.8
a6.5
a6.7
a6.6
A7.6
A8.10
a6.8
d6.5
d6.8
d6.7
d6.6
D7.4
D7.8
D7.7
D7.5
D7.6

67.



C7.11
A7.6
a6.8
d6.5

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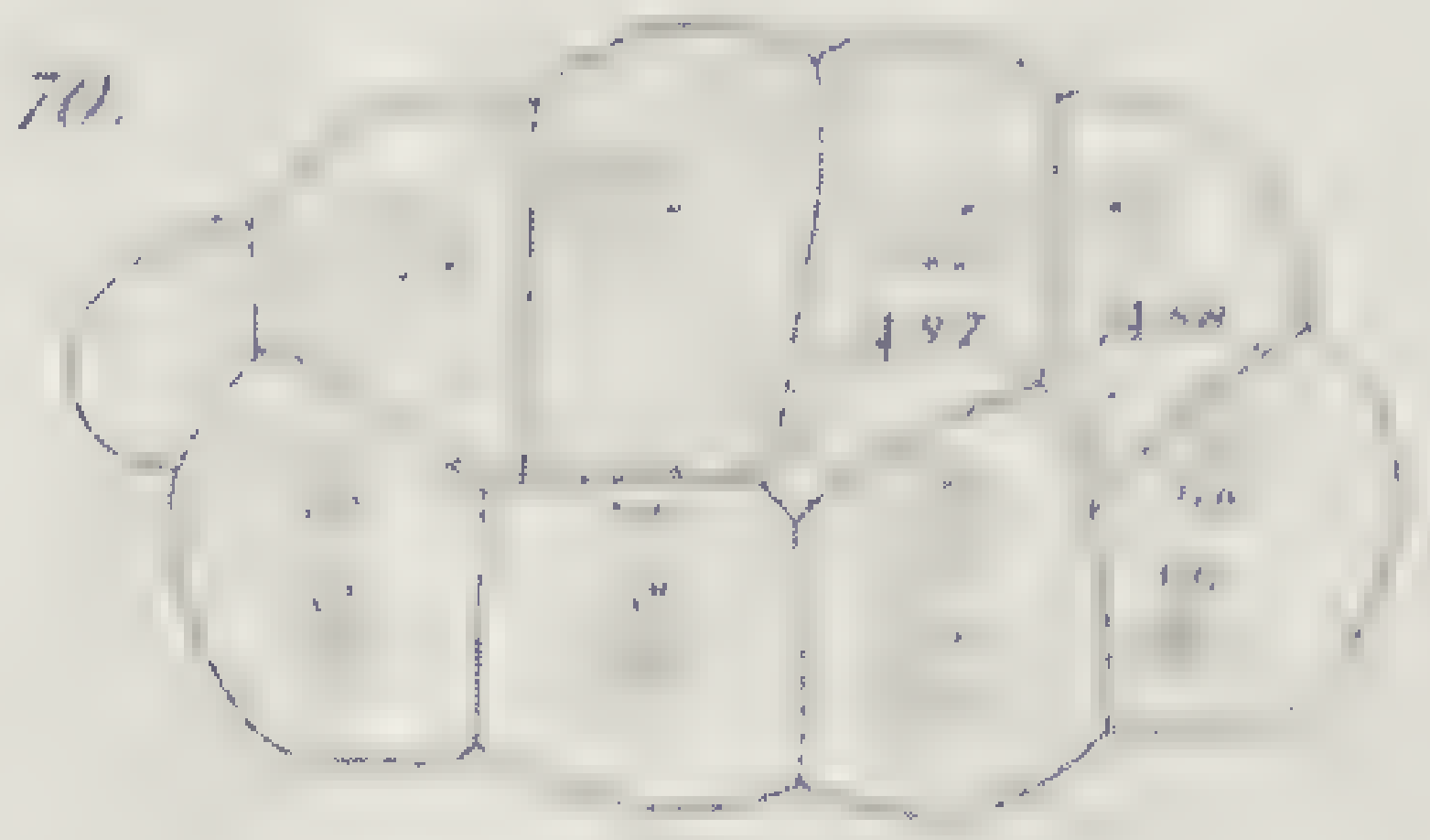

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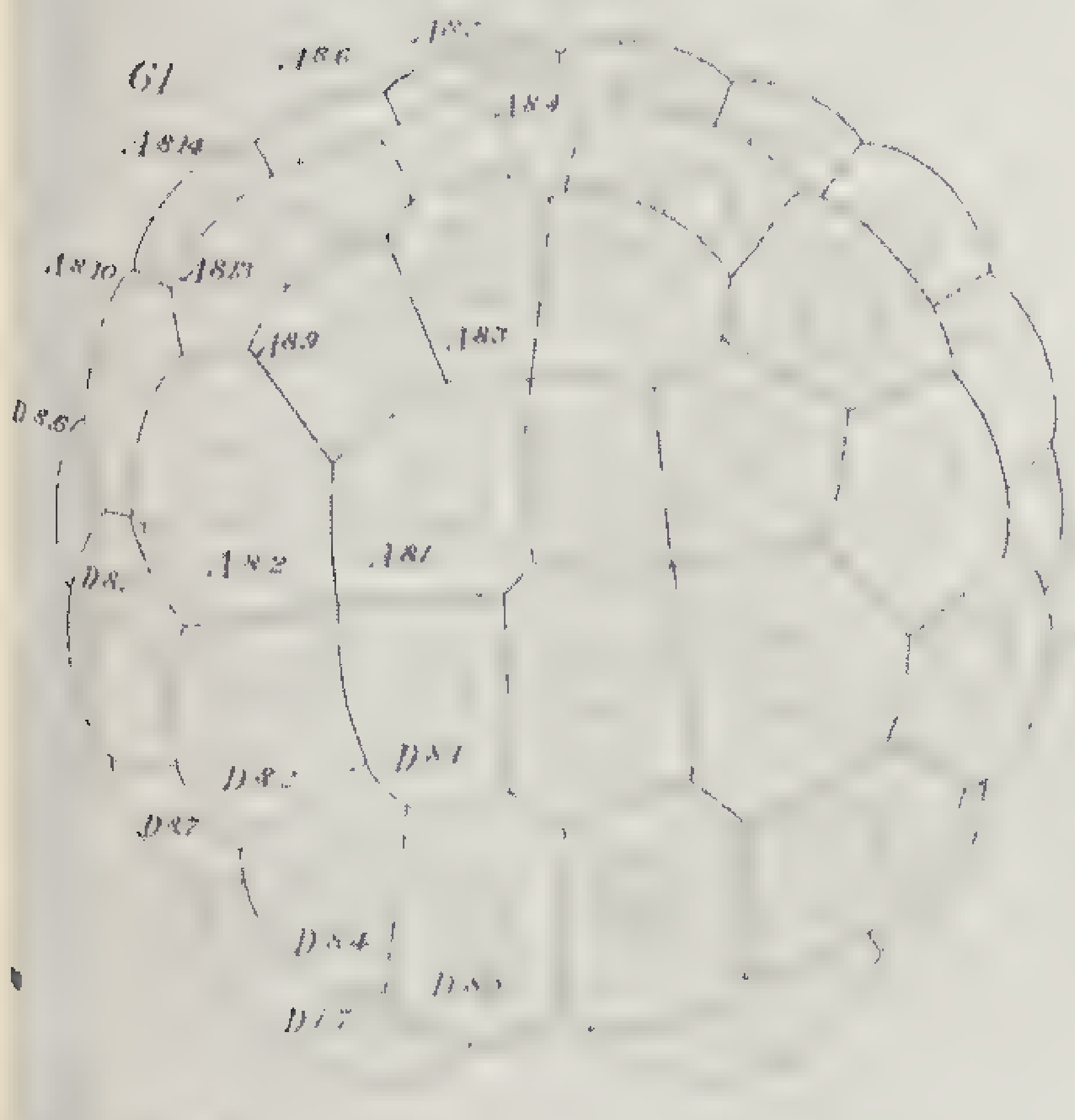
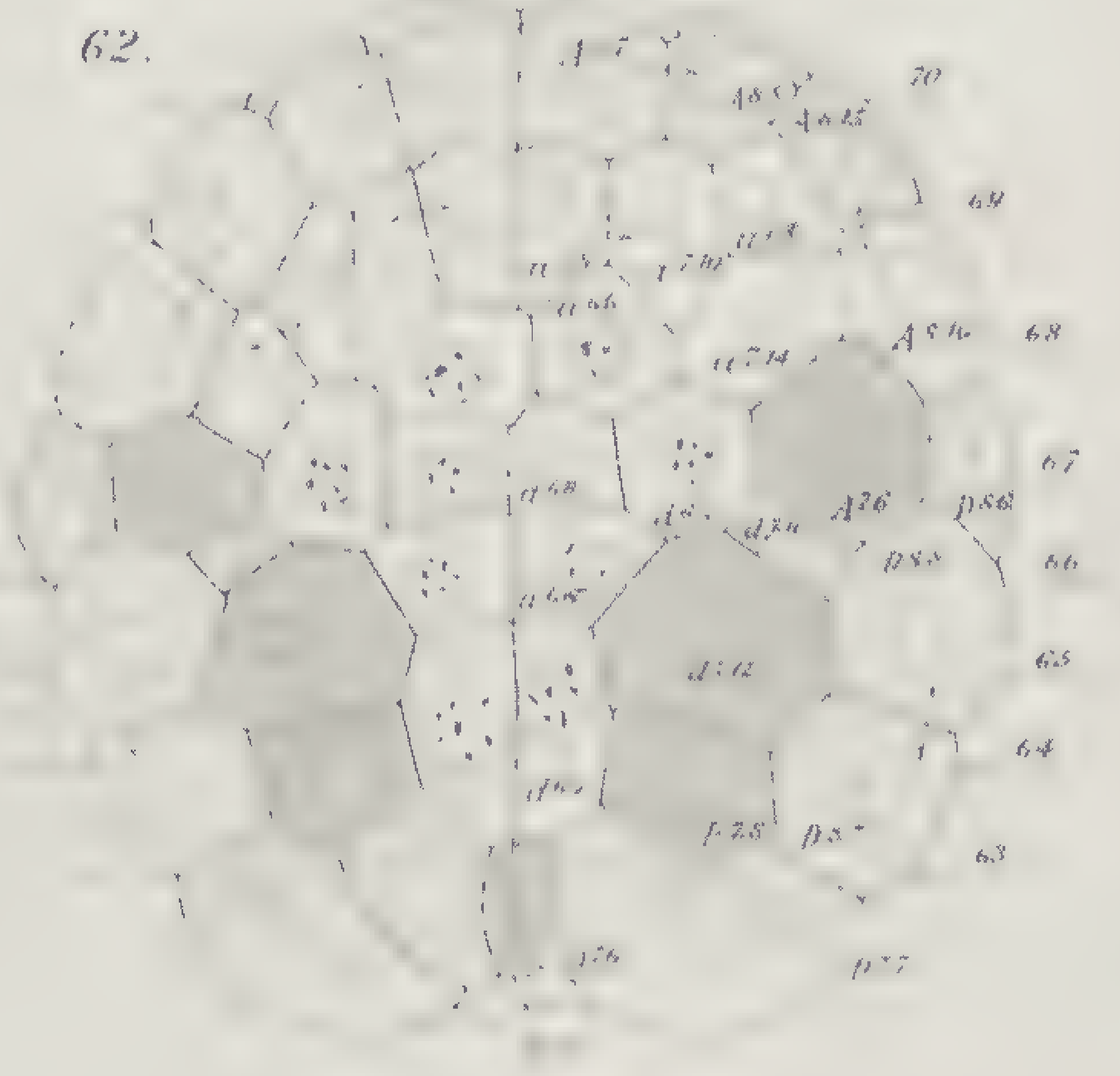


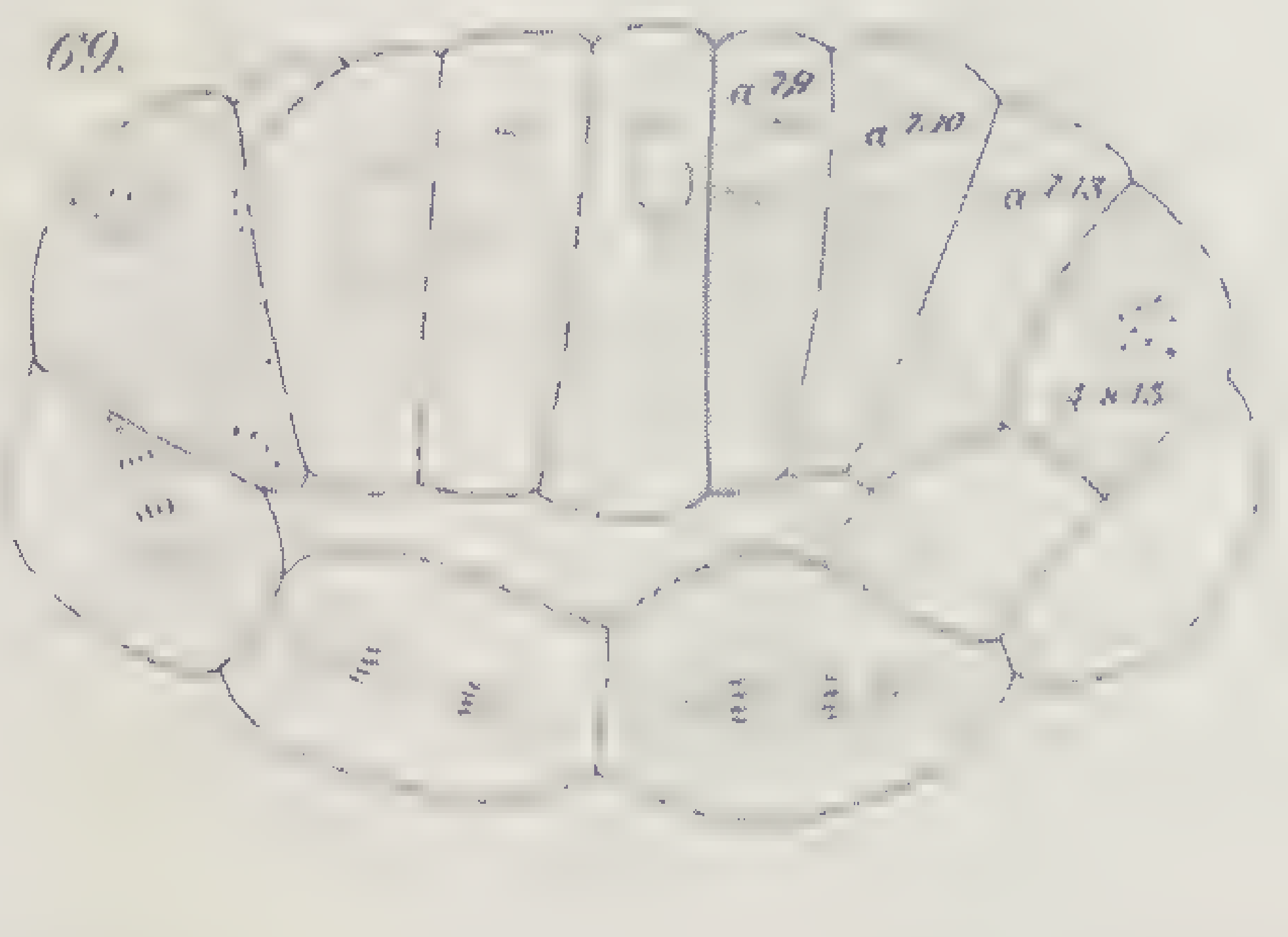
A8.6
A8.5
A8.4
A8.14
A8.10
A8.13
A8.9
A8.3
D8.6
D8.
A8.2
A8.1
D8.1
D8.2
D8.7
D8.4
D8.3
D7.7

62.



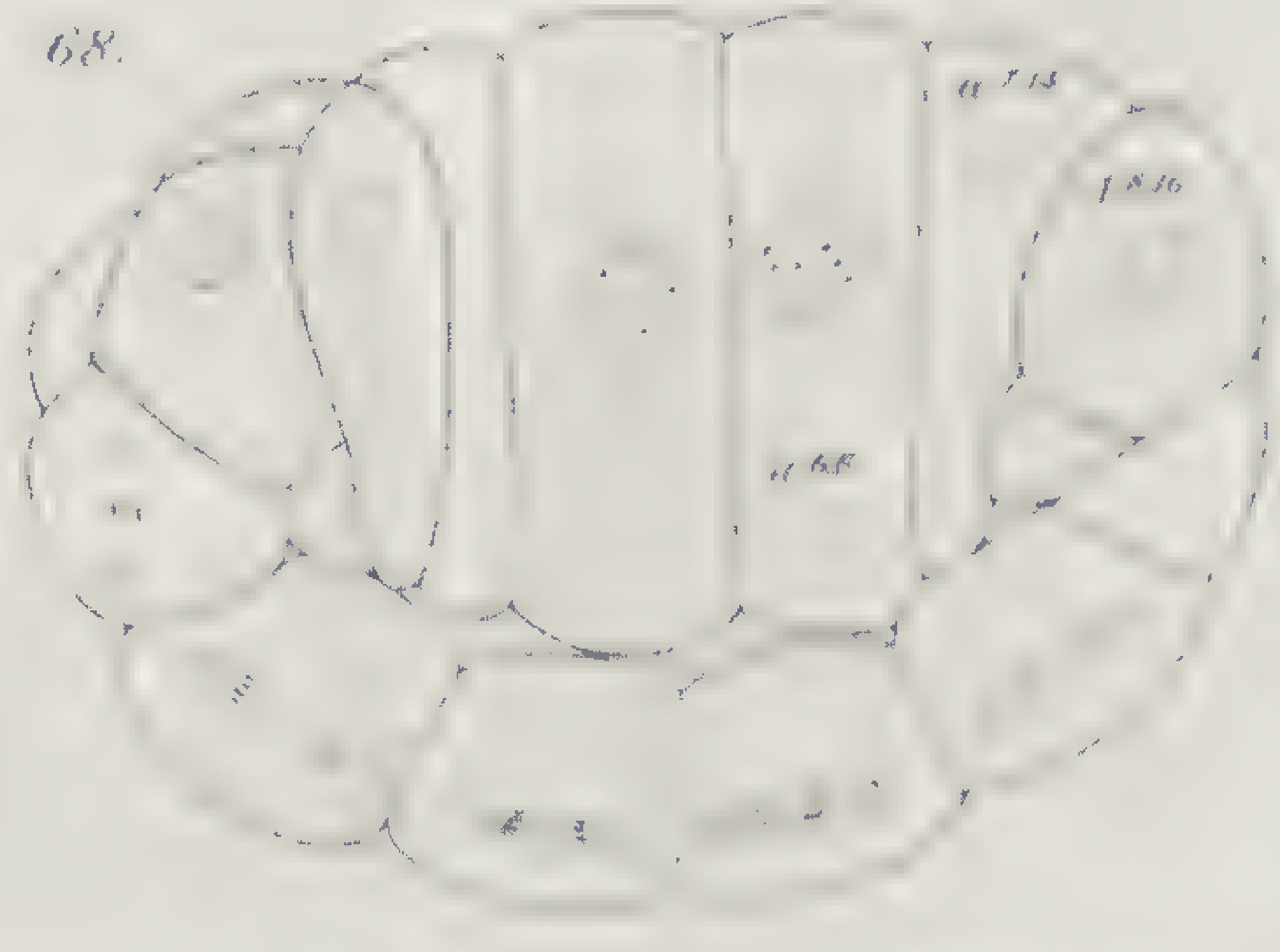
A7.7
A8.15
70
69
68
67
66
65
64
63
A7.6
D8.6
D8.8
D7.8
D7.7

69.



a7.9
a7.10
a7.13
A8.15

68.



a7.13
A8.16
a6.8

PLATE XI.

Magnification of all Figures, 560 diameters.

Fig. 71. 112-cell stage, dorsal view.

Fig. 72. Early gastrula, dorsal view.

Figs. 73–77. Five cross sections through an early gastrula (128-cell stage). For position of sections in embryo, see horizontal lines 73–77 in Fig. 72.

Note. — Consult general statement regarding lettering under Explanation of Plates.

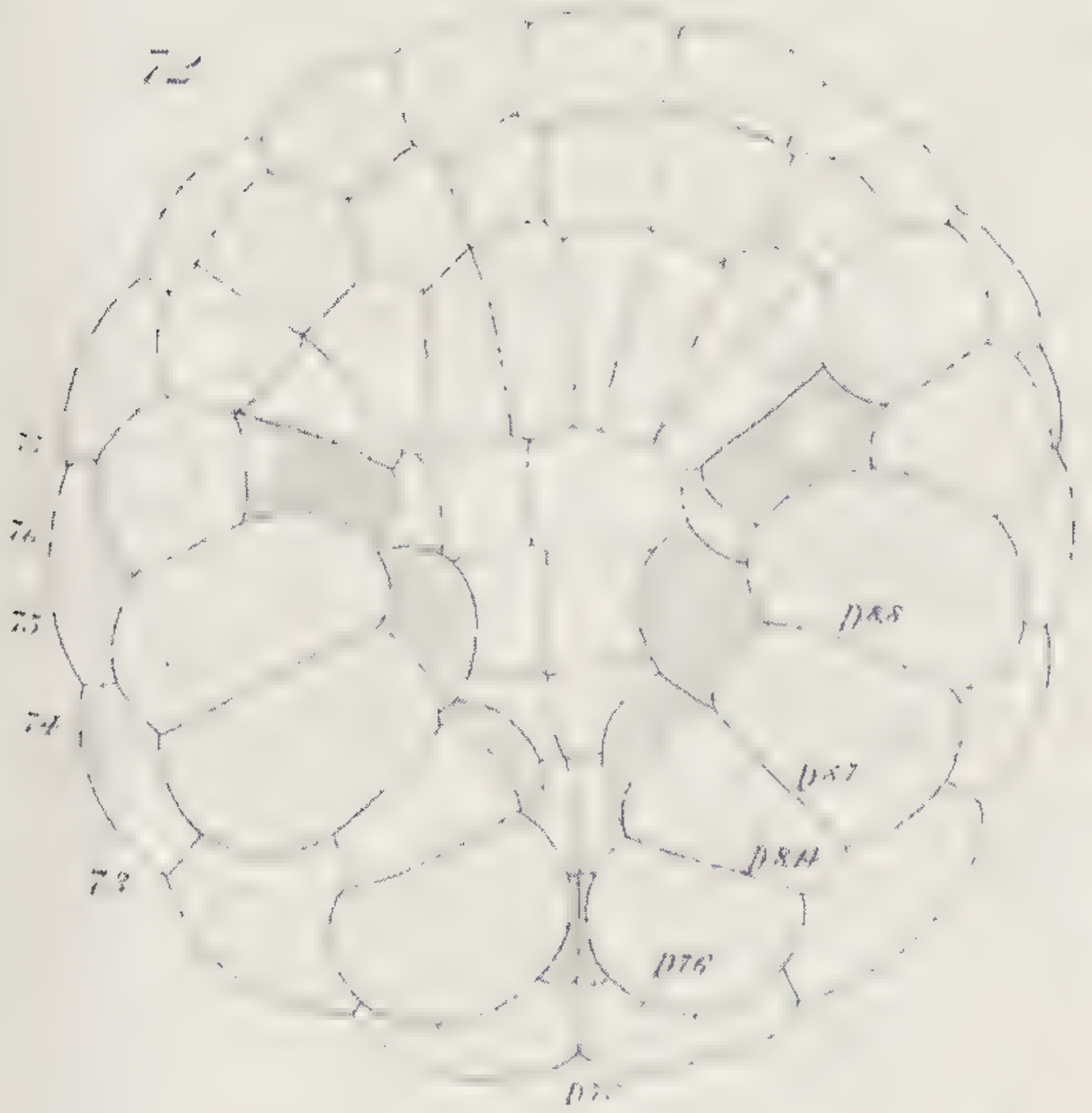
Fig. 78. Sagittal section through an early gastrula (older than that shown in Fig. 72).

Fig. 79. Similar section through a slightly older stage.

Fig. 80. Surface (dorsal) view of late gastrula.

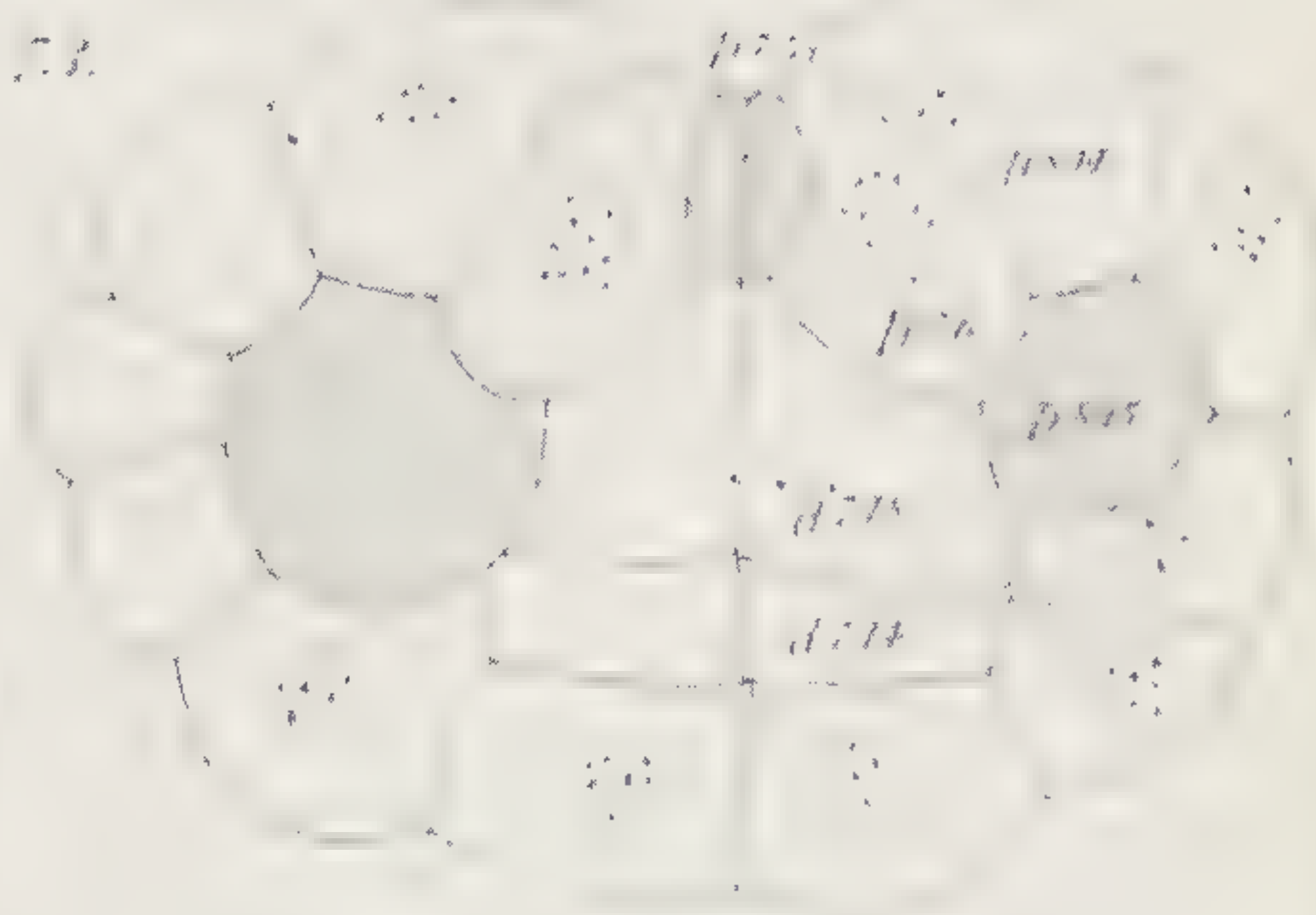
Figs. 81–83. Three horizontal sections from a series through a late gastrula.

72



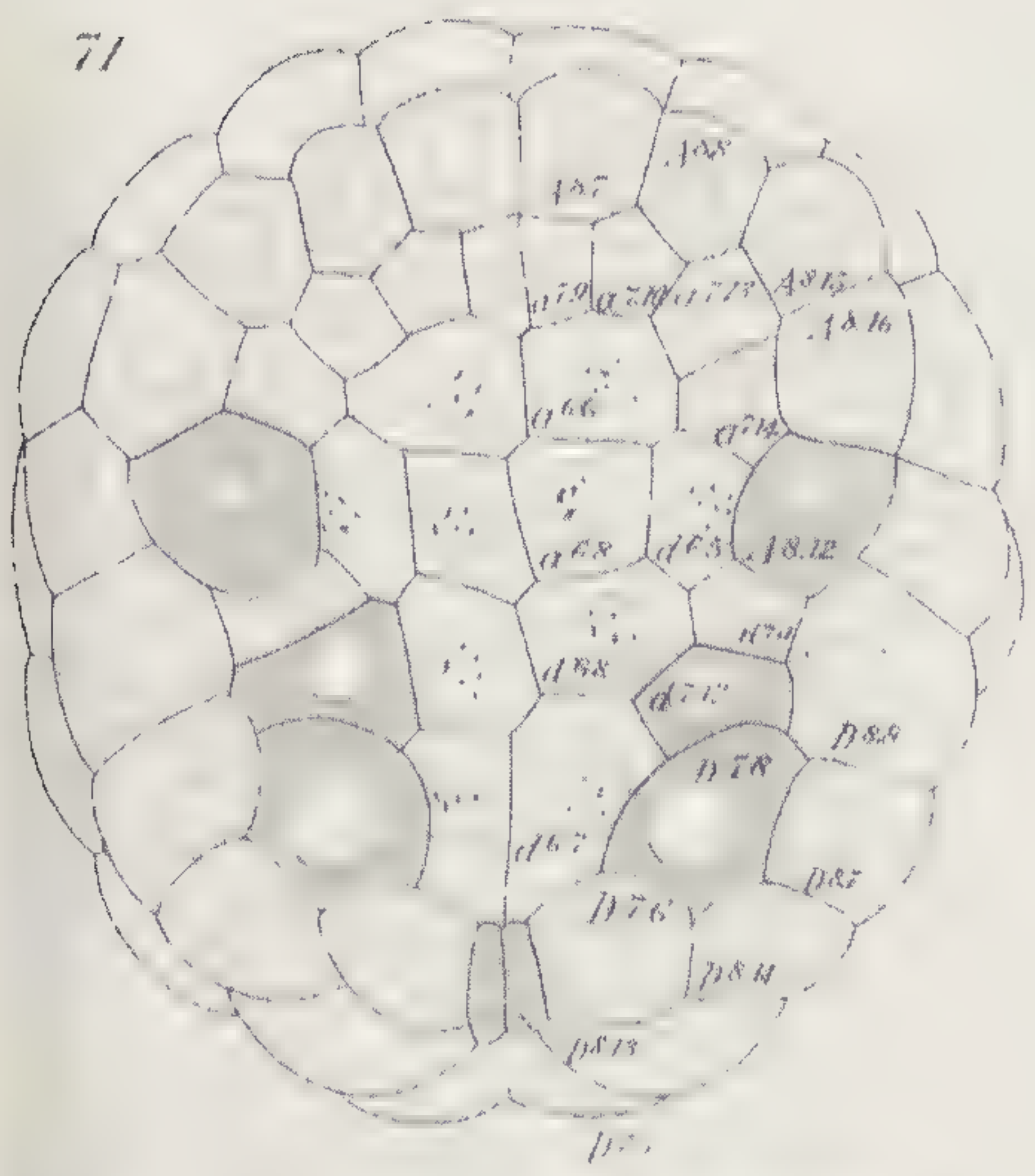
77
76
75
74
73
D8.8
D8.7
D8.11
D7.6
D7.5

73.



D7.5
D8.14
D7.6
D8.15
d7.15
d7.14

71



A8.7
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a7.10
a7.12
A8.15
A8.16
a6.6
g
a7.14
a6.8
d6.5
A8.12
d7.9
d8.8
d7.12
D7.8
D8.9
d6.7
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D7.6
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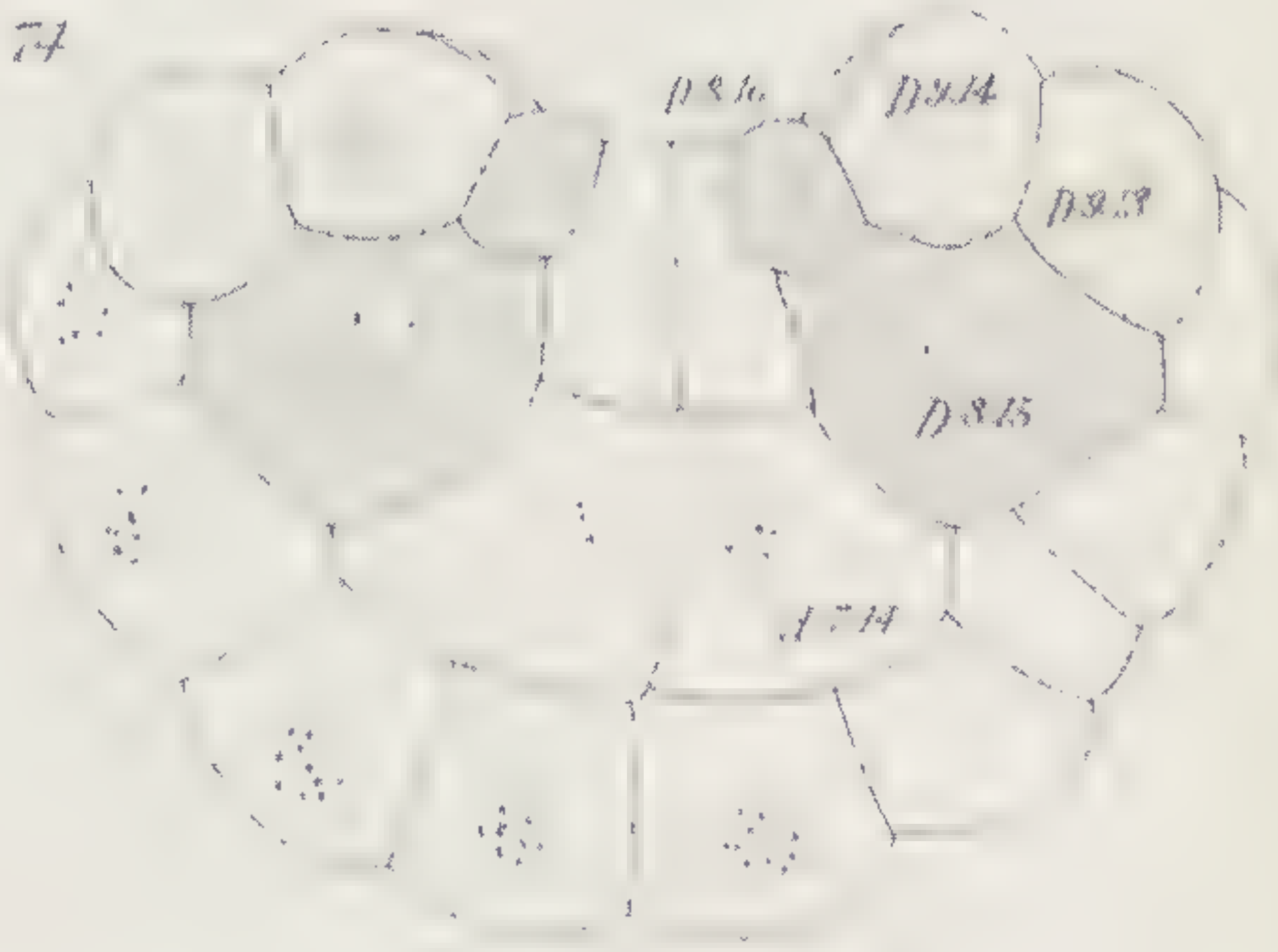
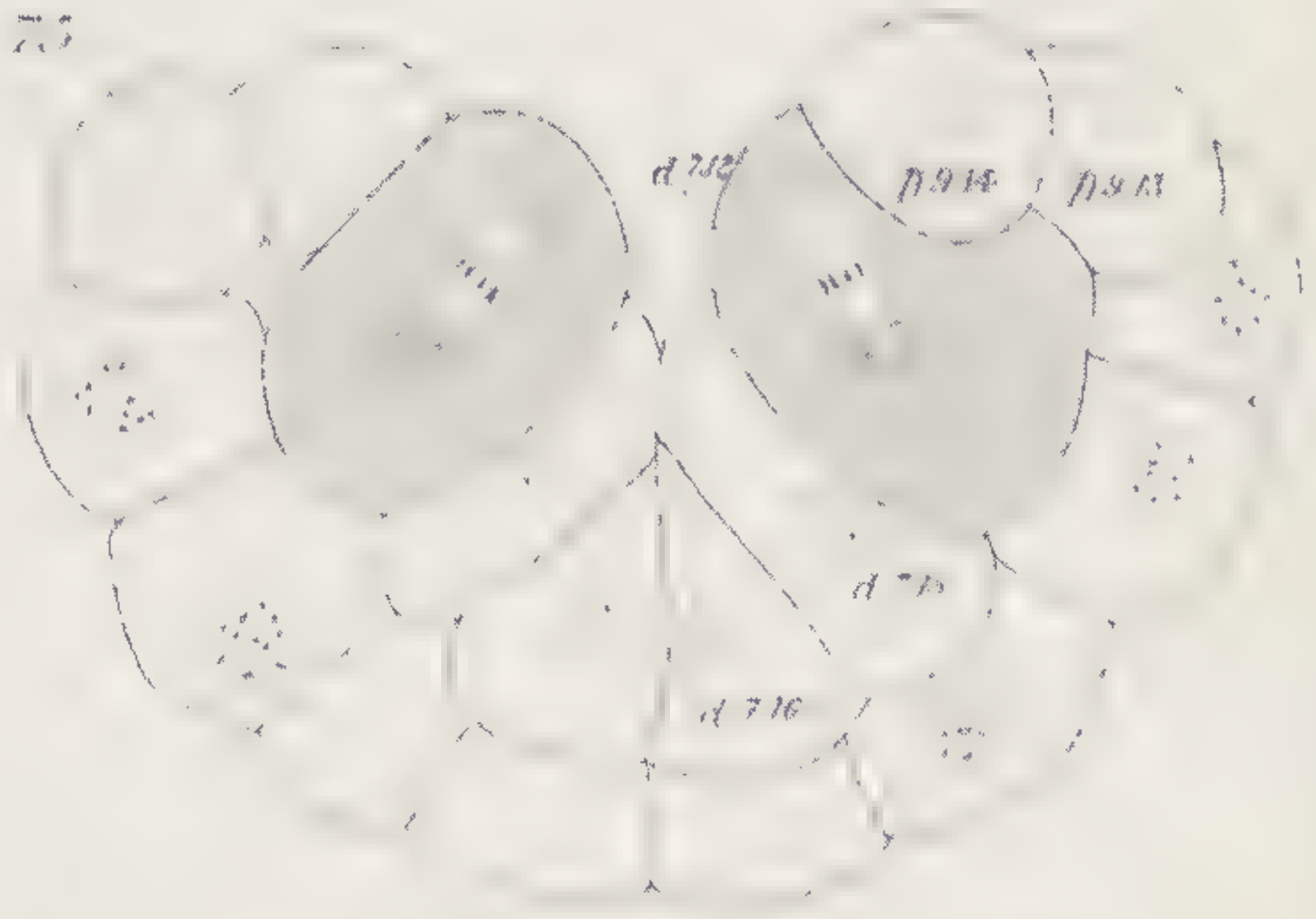
D7.5

74



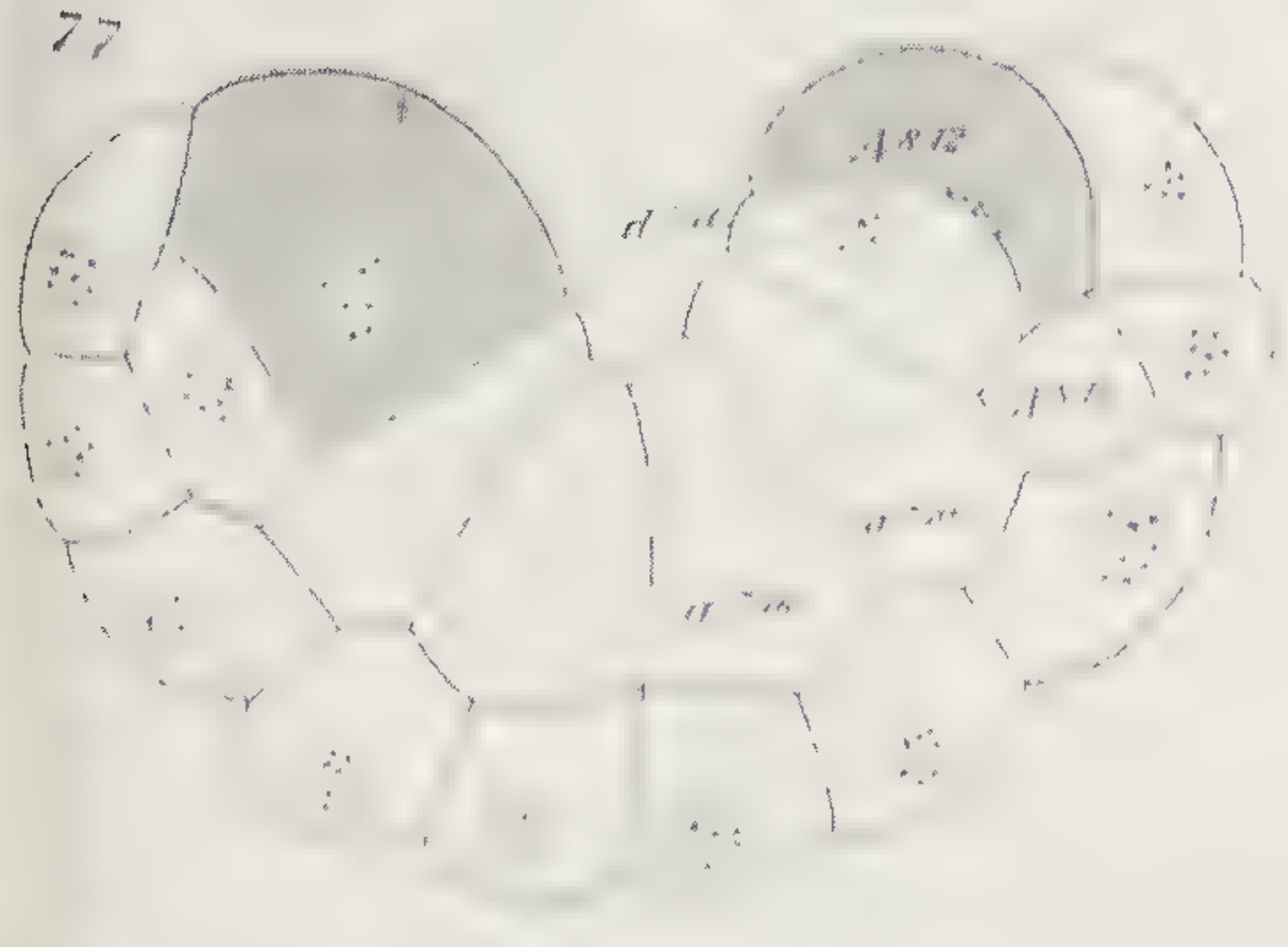
D8.16
D9.14
D9.3
D8.15
d7.14

75



d7.12
D9.14
D9.13
d7.15
d7.16

77



A8.12
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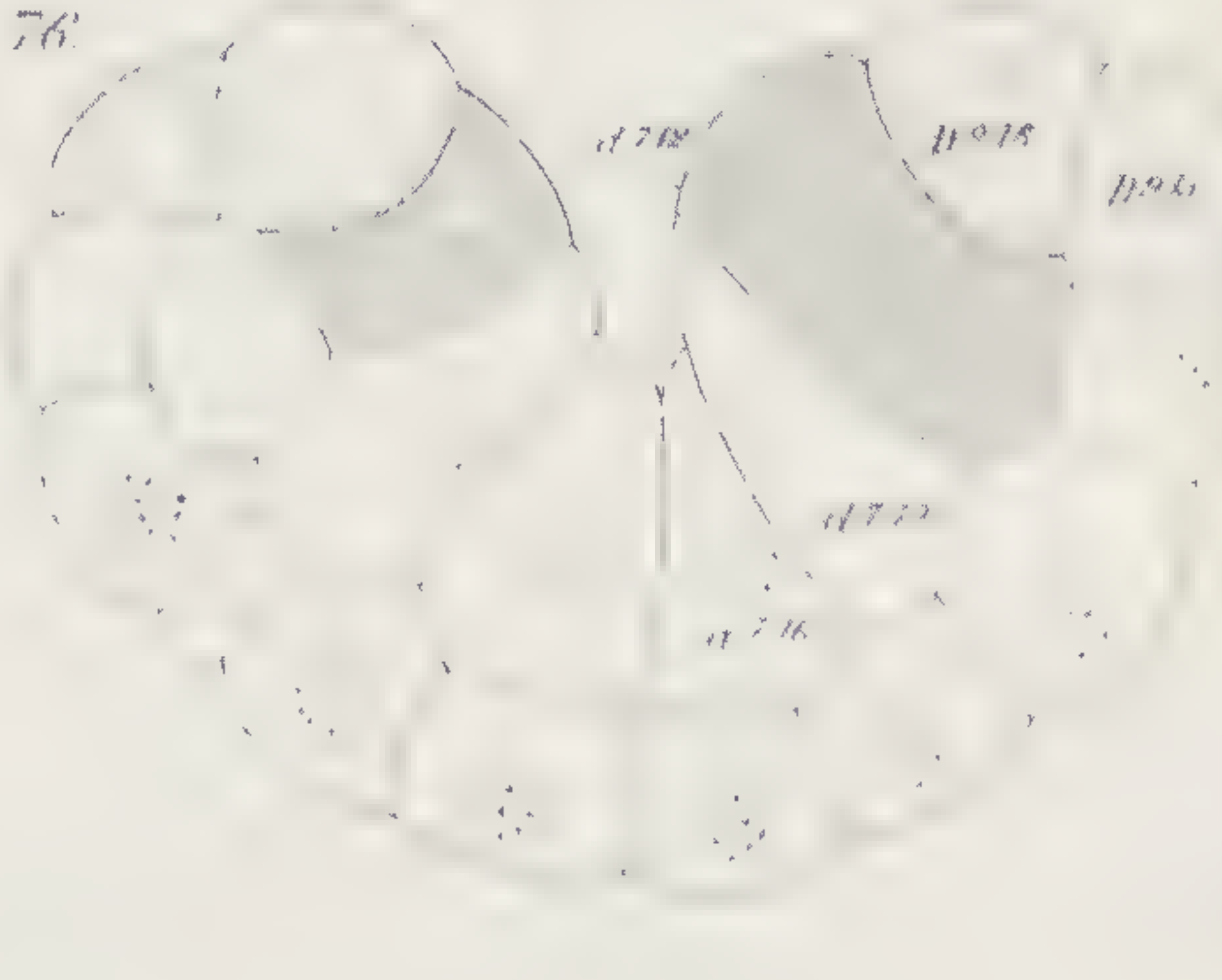
a7.10
a7.16

76.



d7.12
D9.13
D9.6
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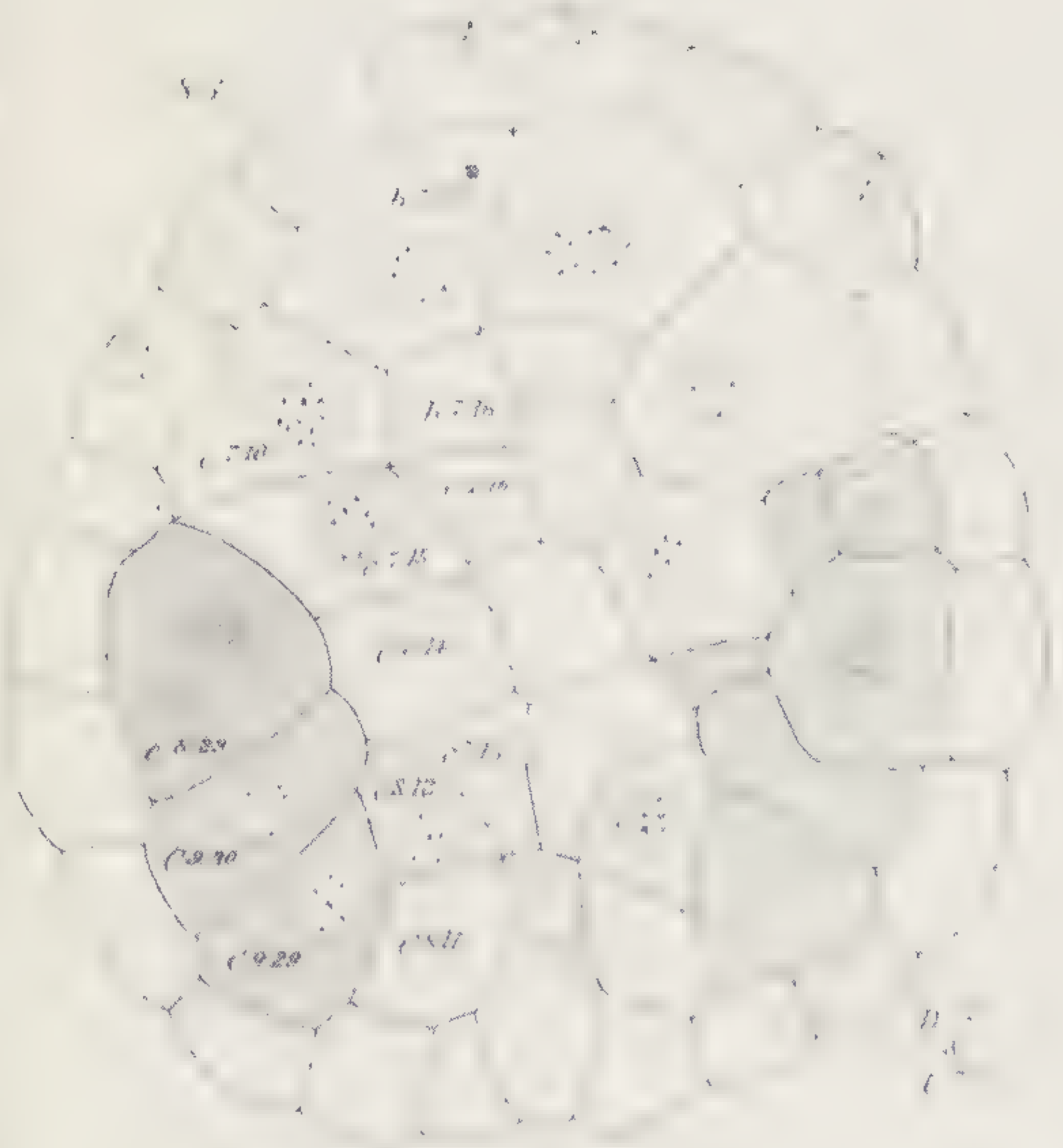
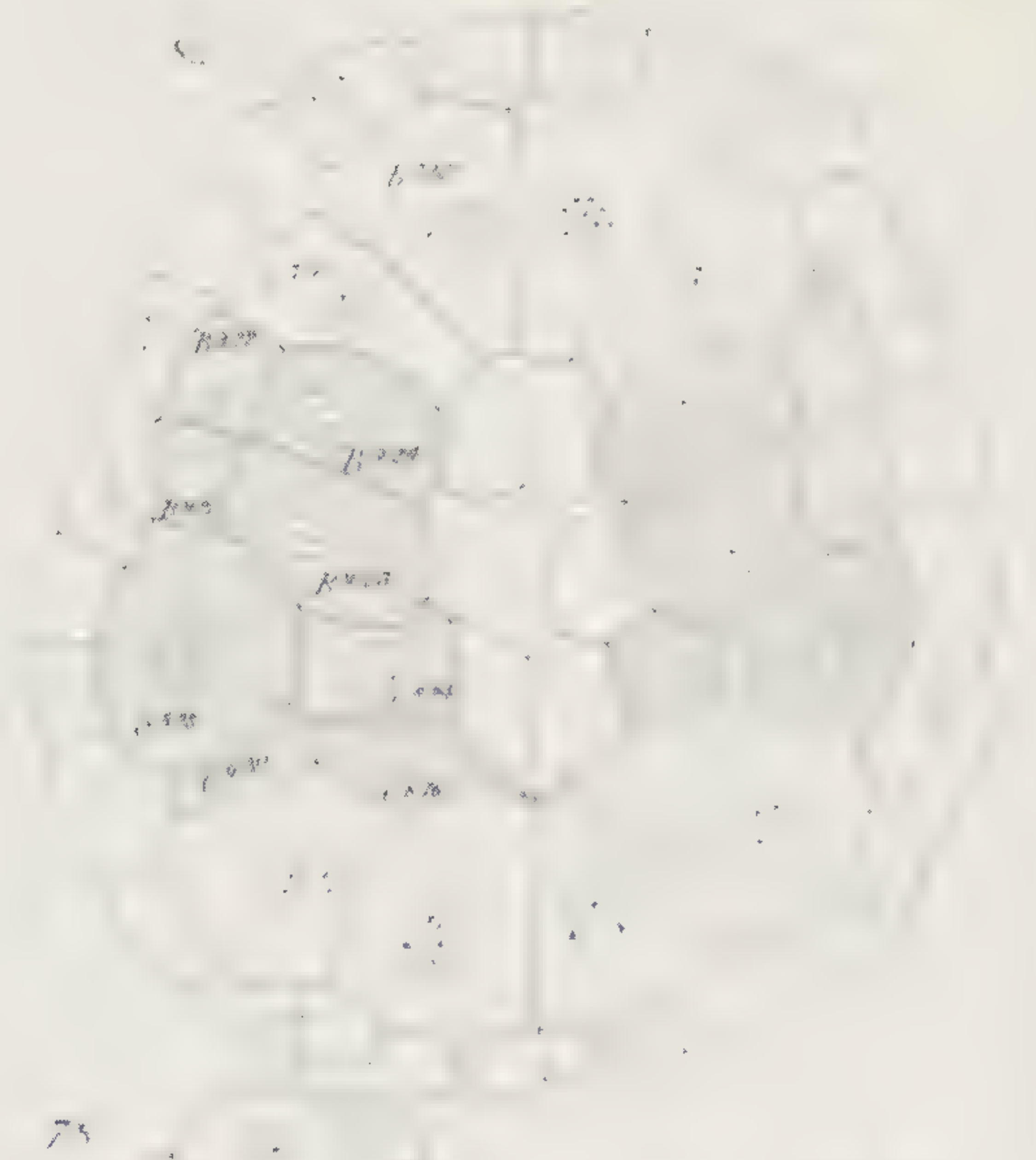
a7.16





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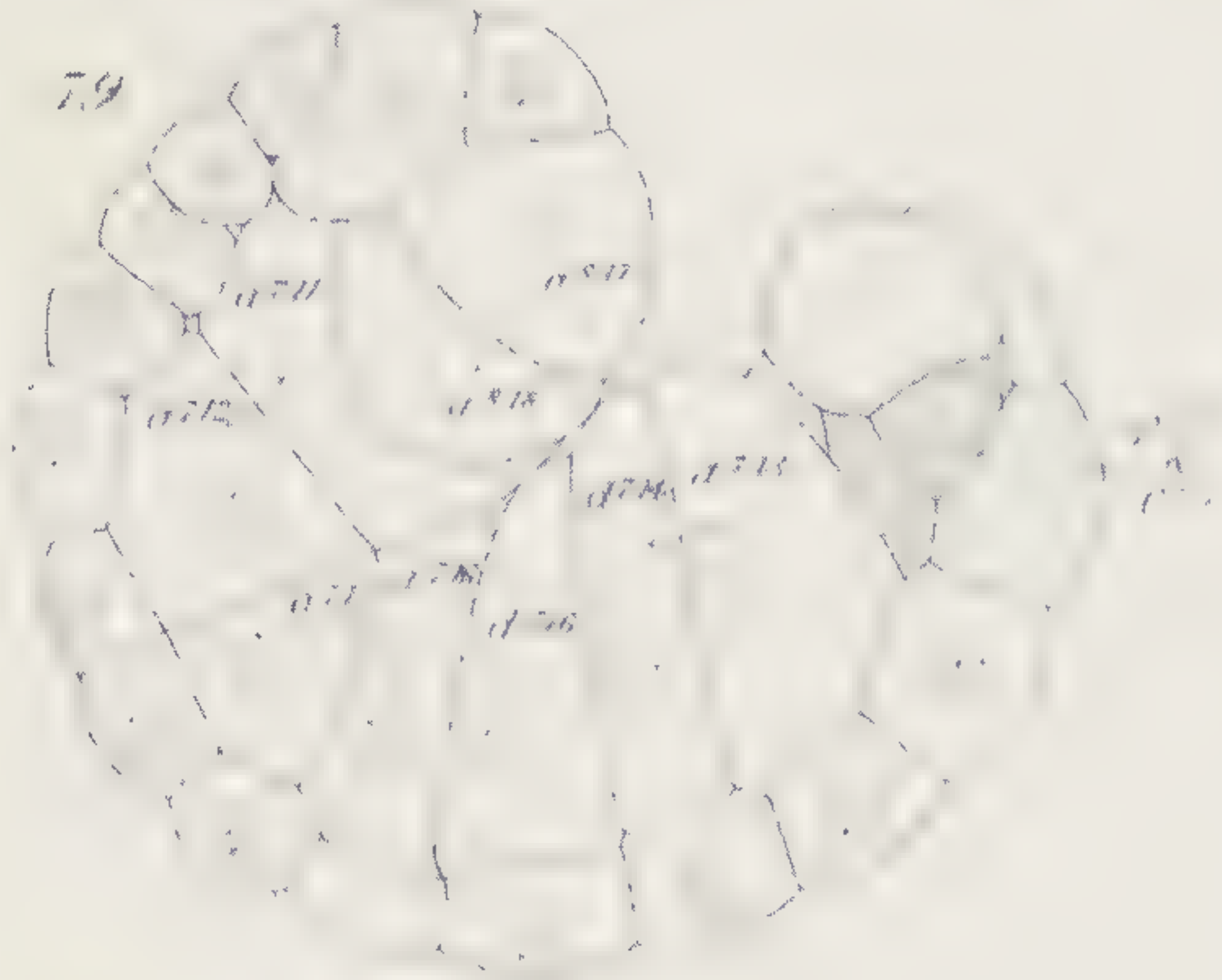

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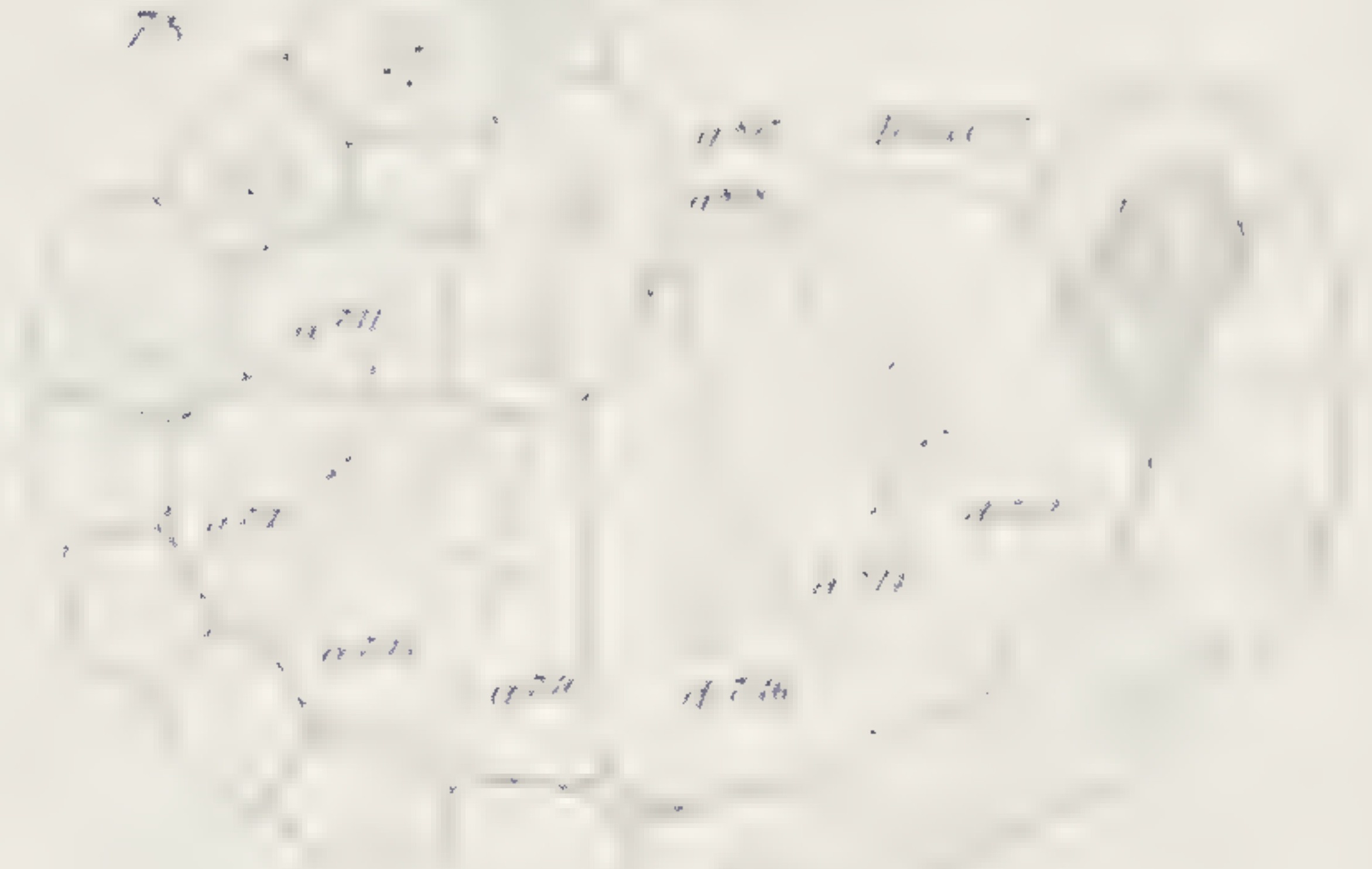
a7.15
d7.16

78



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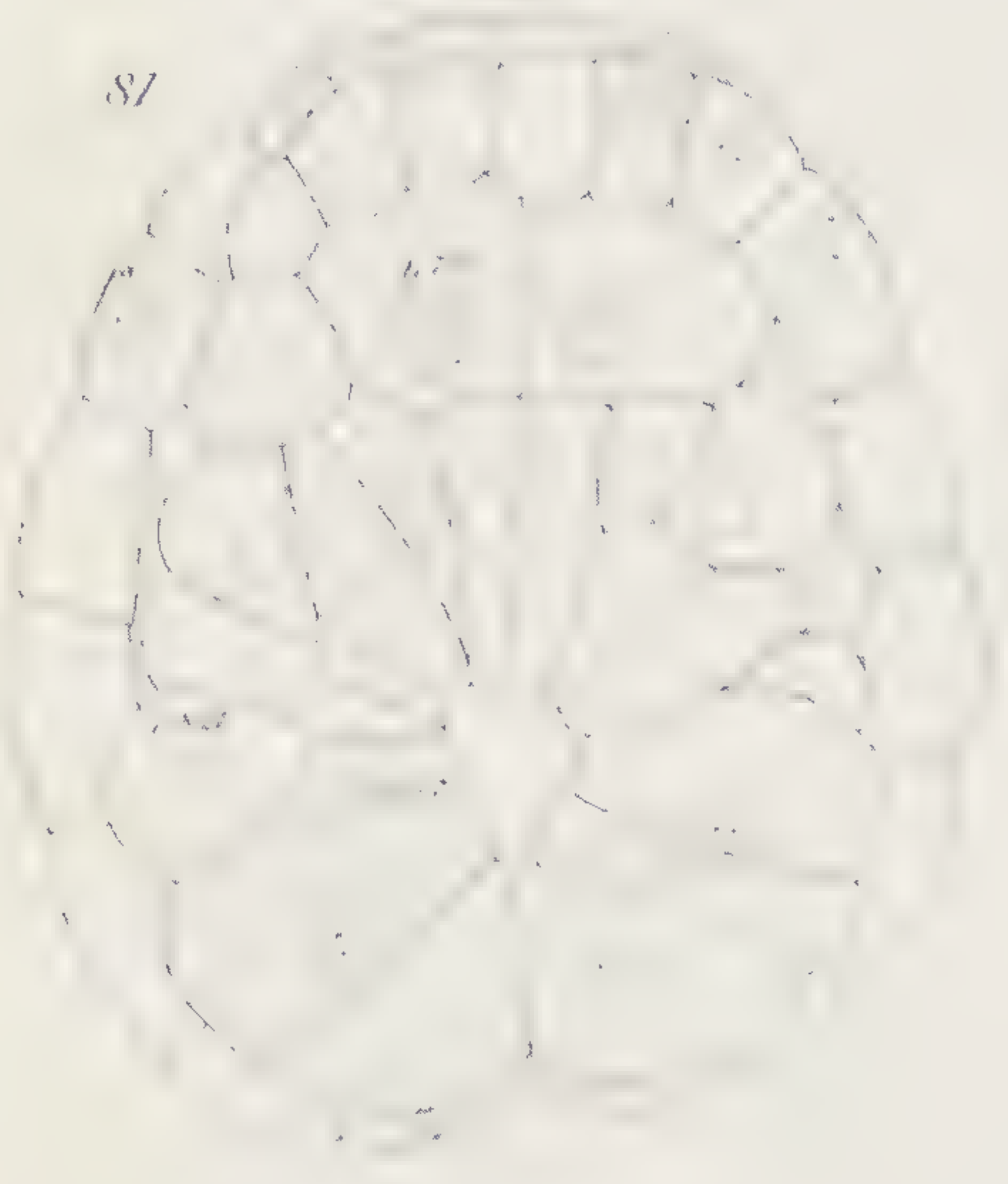

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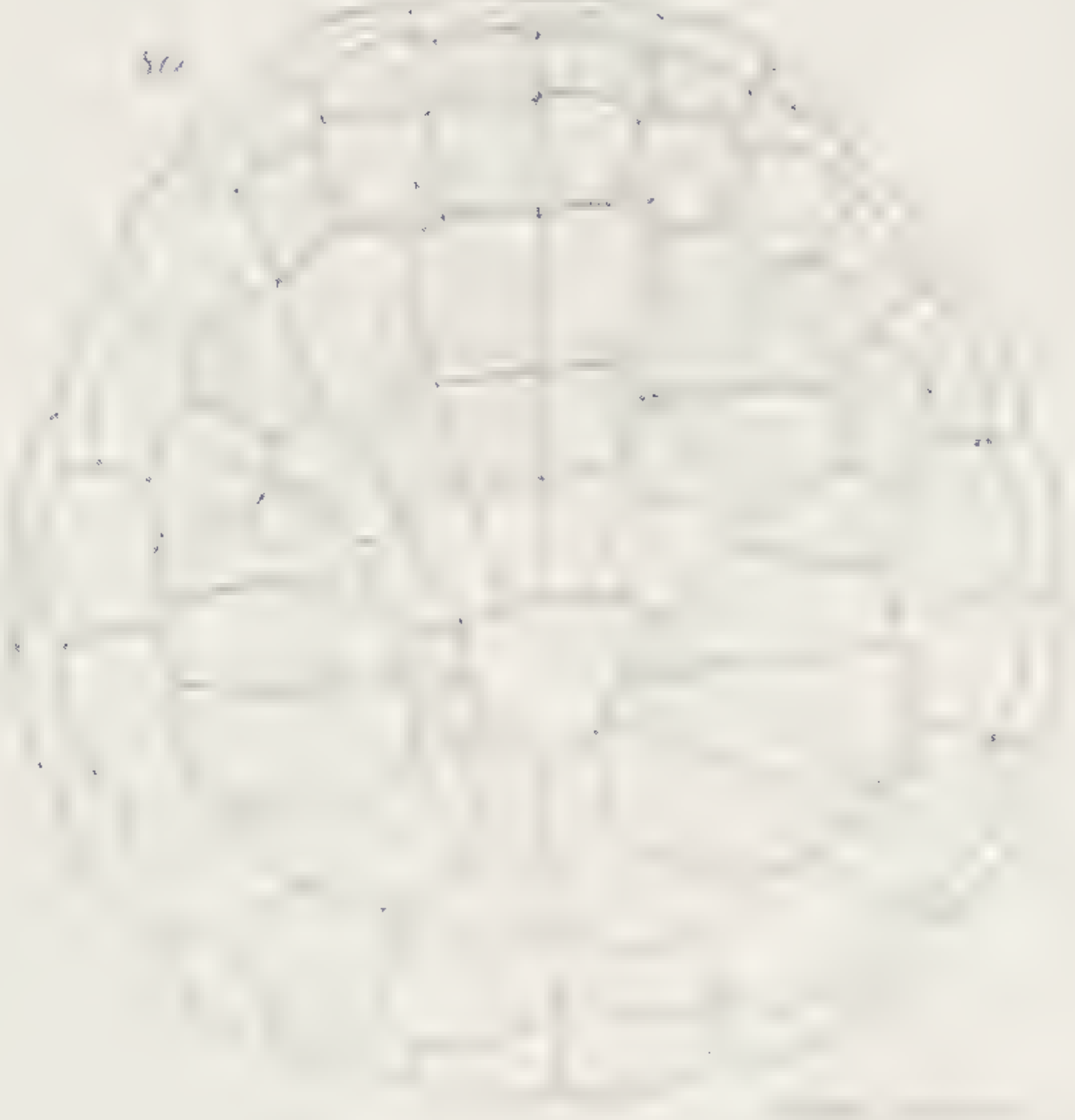


PLATE XII.

Magnification of all Figures, 560 diameters.

Figs. 84–87. Four cross sections from a series through a gastrula with wide-open blastopore.

Fig. 84. Section posterior to the blastopore.

Fig. 85. Section through the posterior portion of the blastopore.

Fig. 86. Section through the anterior portion of the blastopore.

Fig. 87. Section anterior to the blastopore.

Figs. 88–92. Five cross-sections through an embryo with greatly contracted blastopore; Figs. 88 and 89 posterior to blastopore; Figs. 91 and 92 anterior to blastopore.

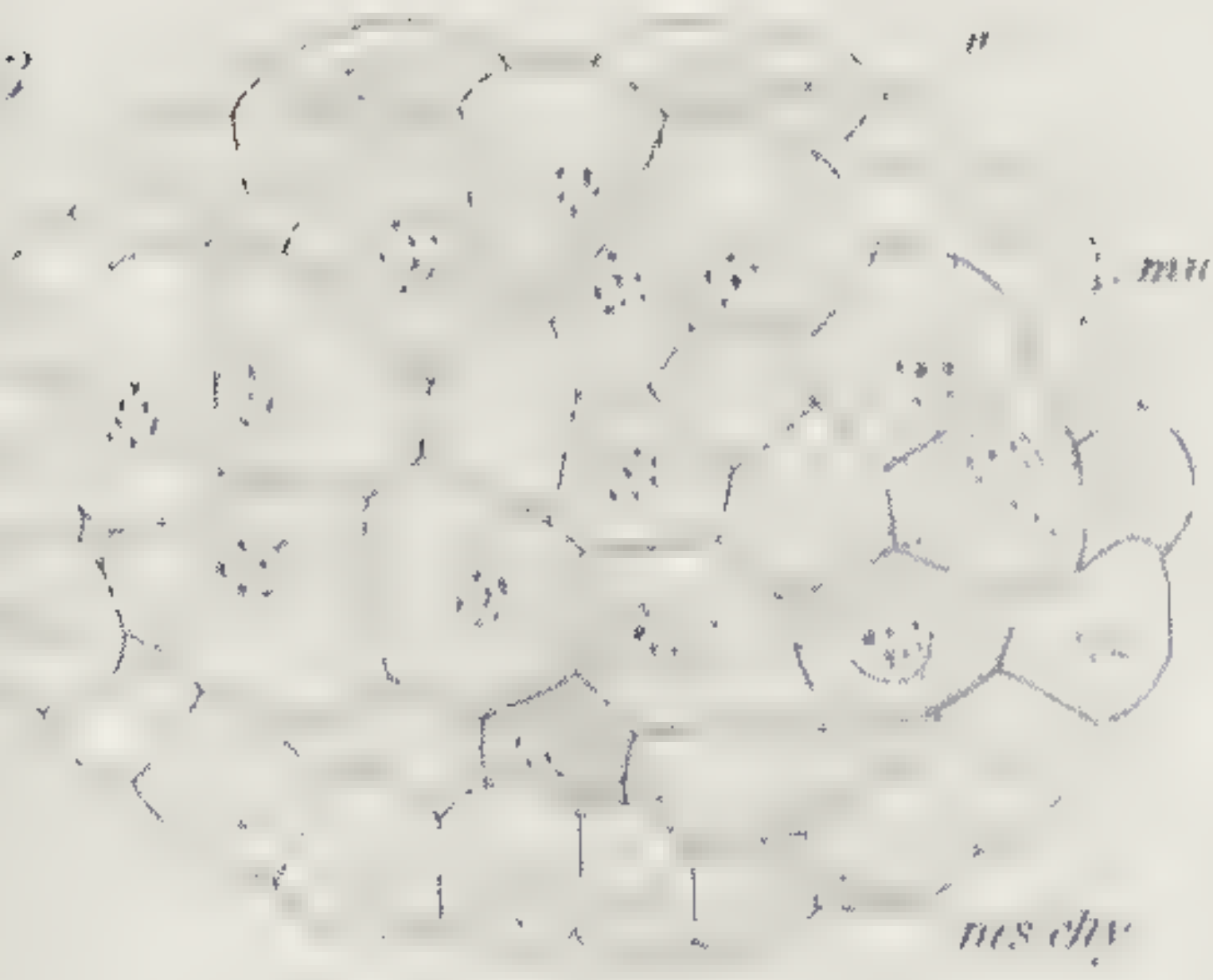
Note. — The large unstippled cell in the left half of Fig. 88 should have been stippled.

Figs. 93–97. Five cross sections through a slightly older embryo. Compare vertical lines 93–97 in Fig. 98. Fig. 93, section posterior to blastopore; Figs. 95–97, sections anterior to blastopore.

Note. — The large unstippled cell in the left half of Fig. 93 should have been stippled, likewise two large cells situated laterally in Fig. 95, one in either half of the Figure.

Fig. 98. Sagittal section through an embryo with nearly closed blastopore.

93.



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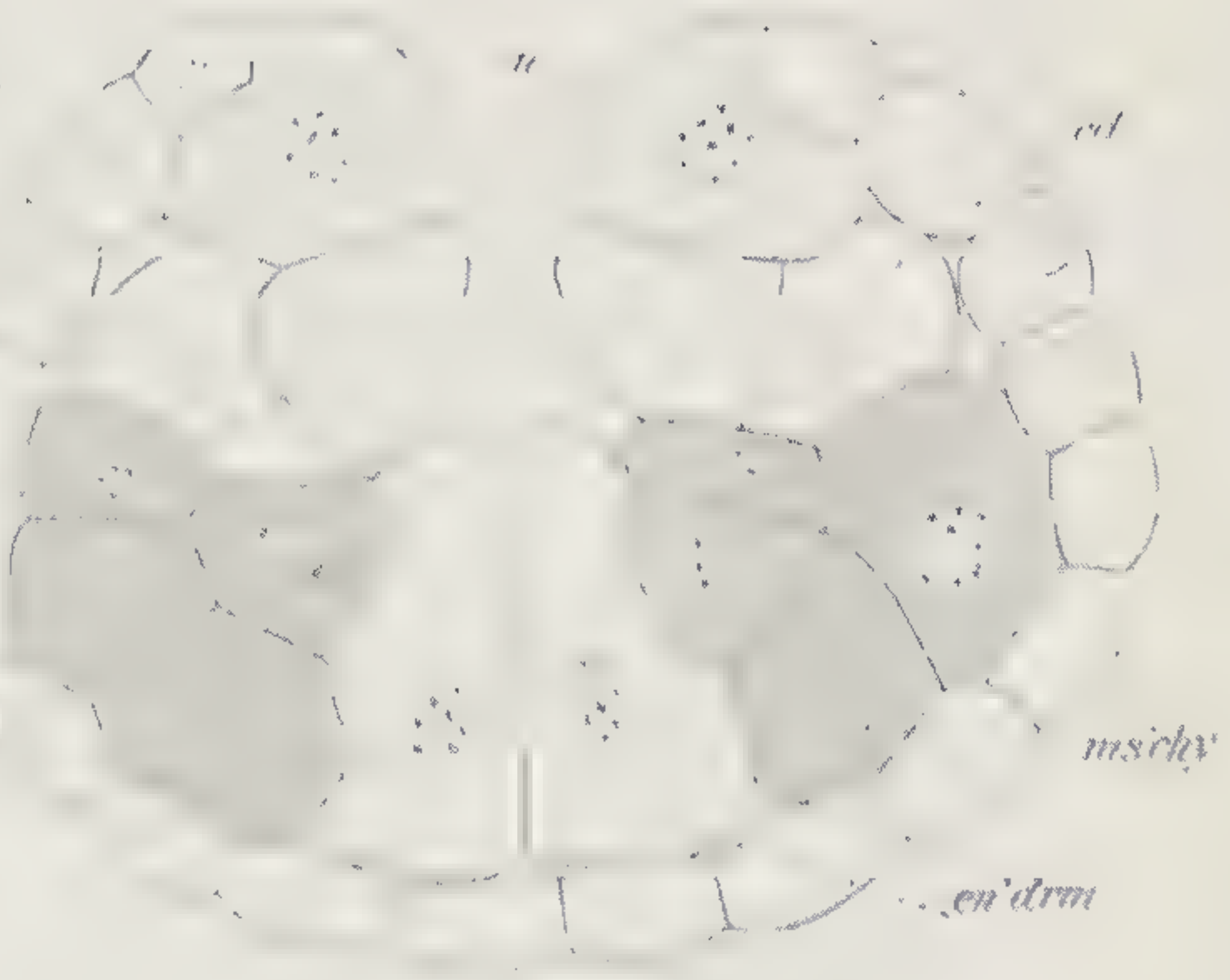
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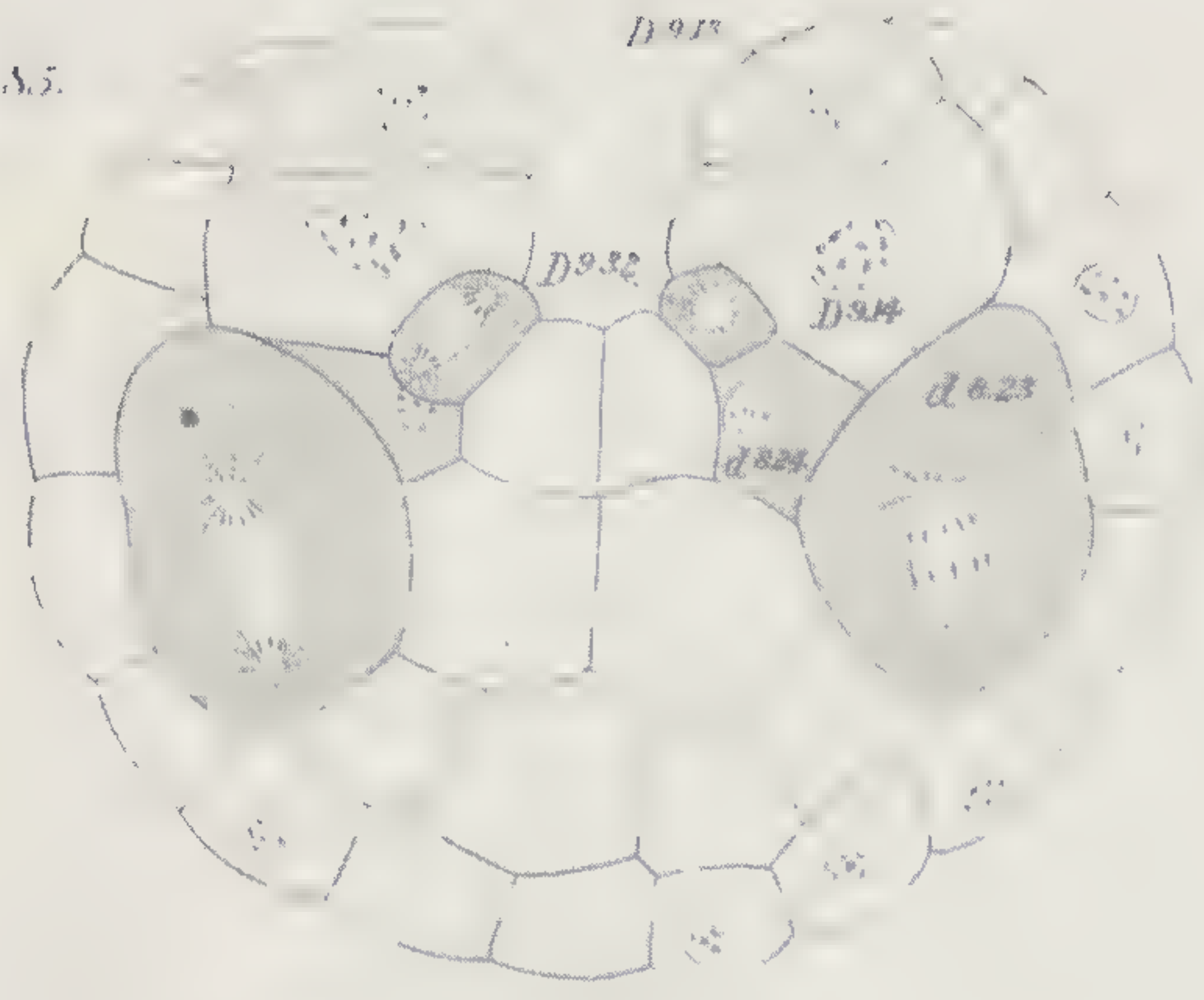
D 9.13
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d 8.23
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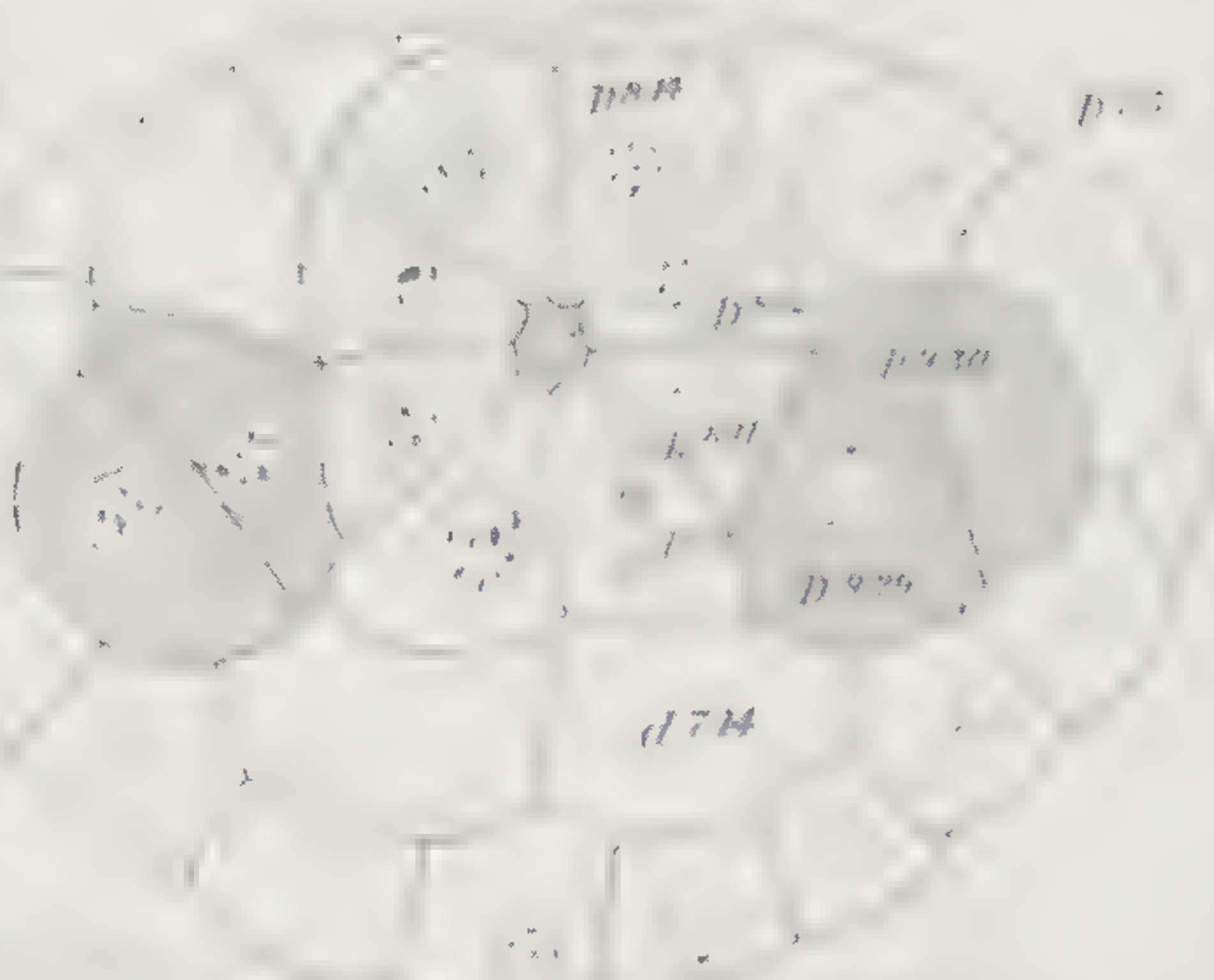
84.



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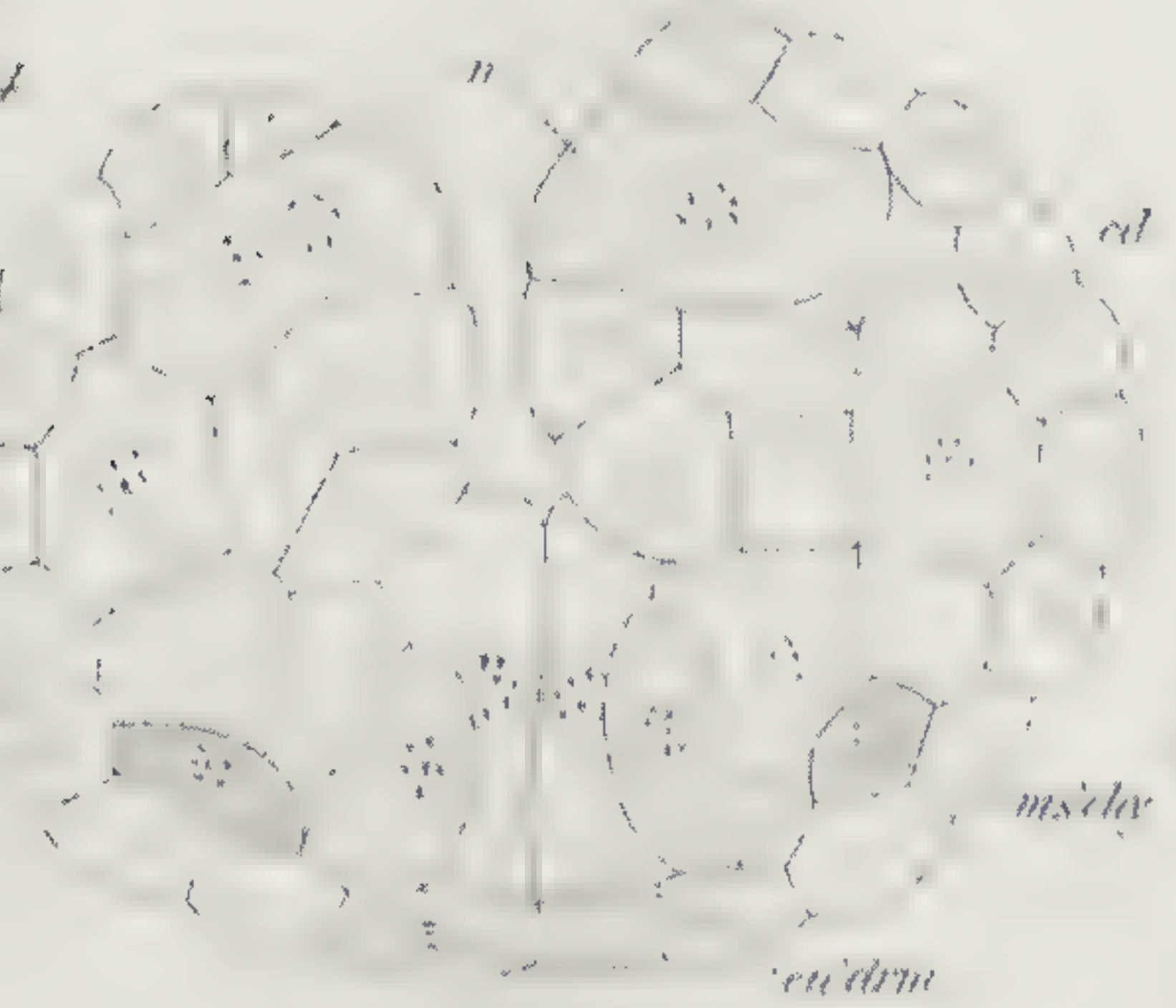
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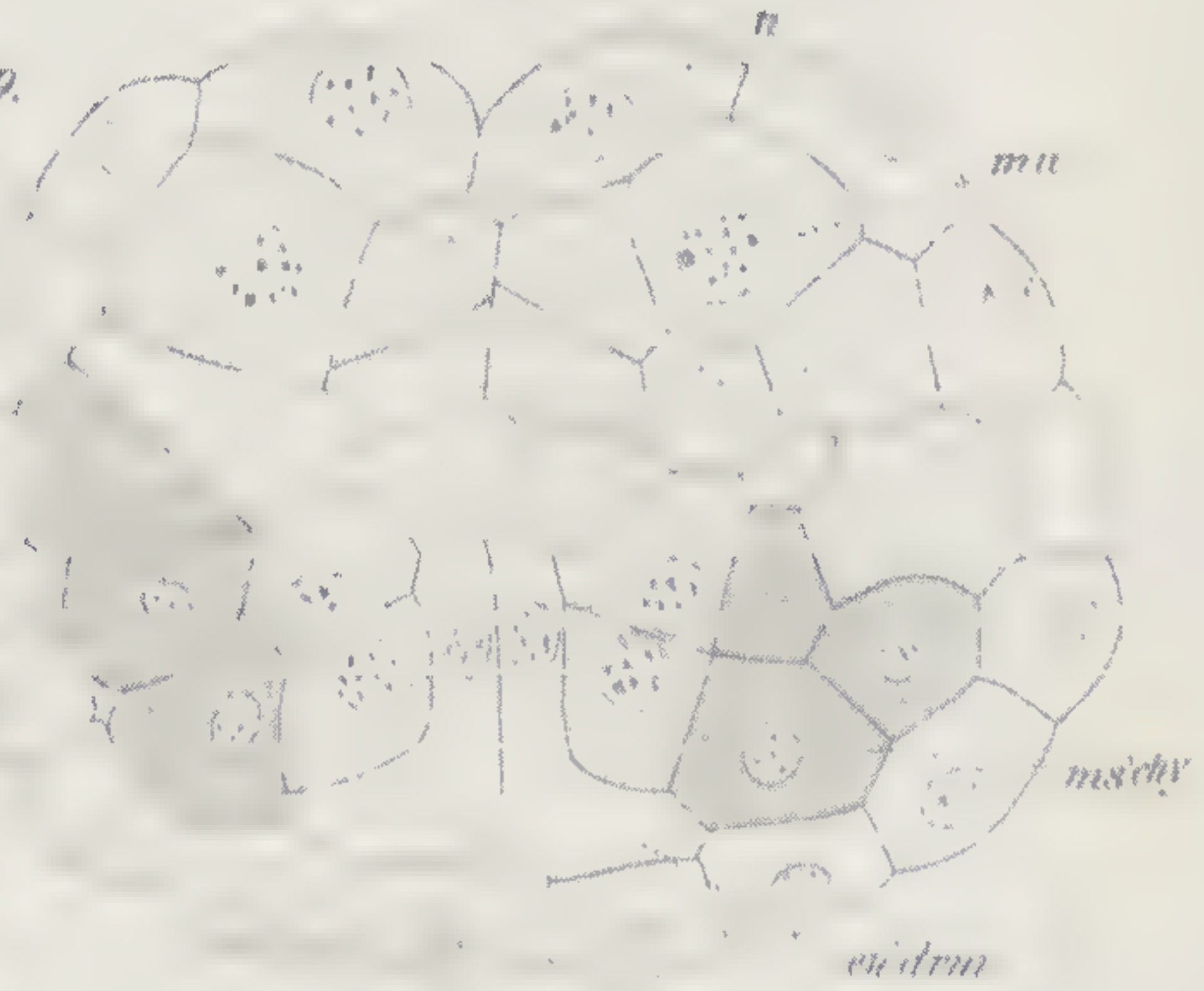
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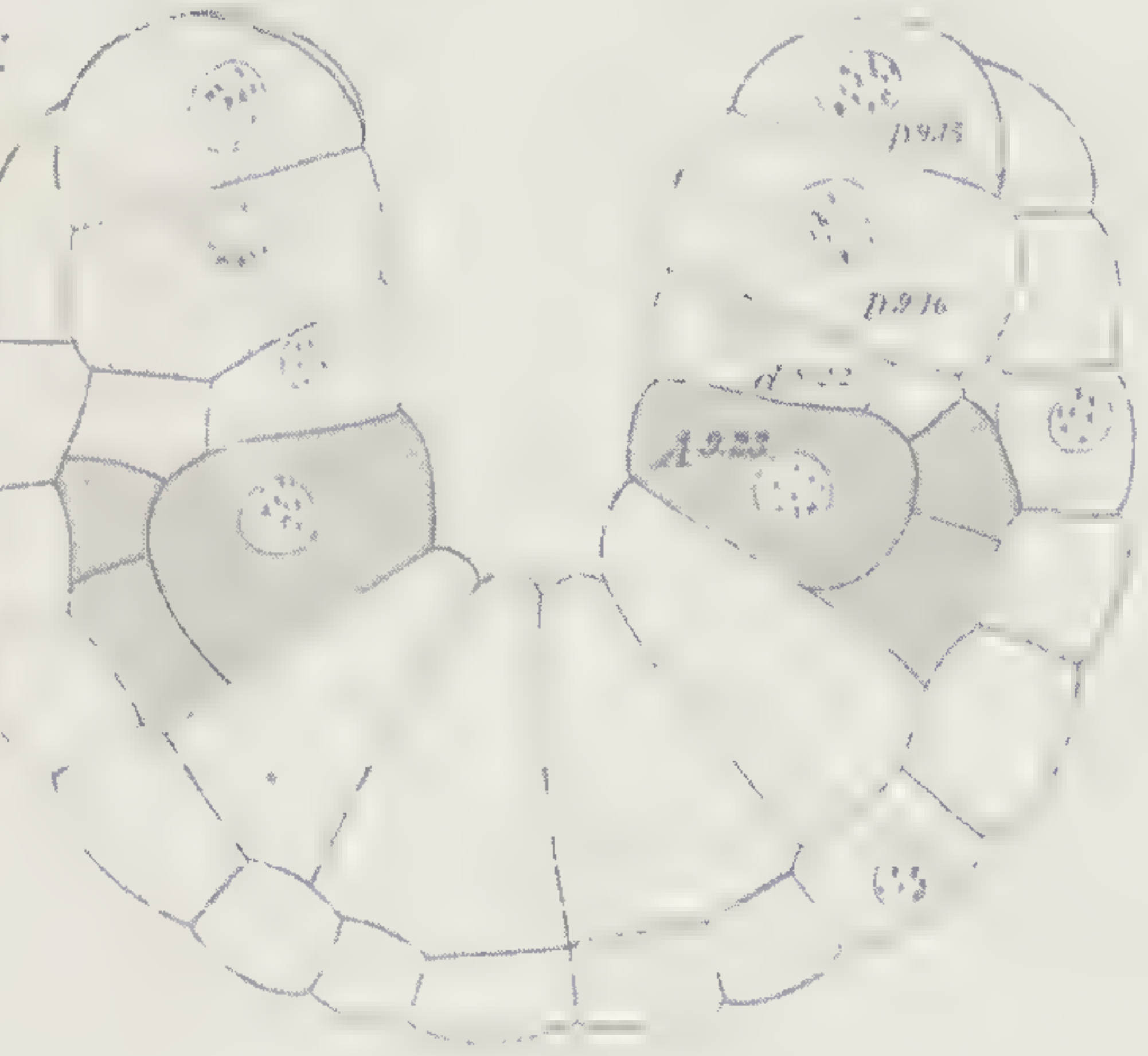
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A 9.23

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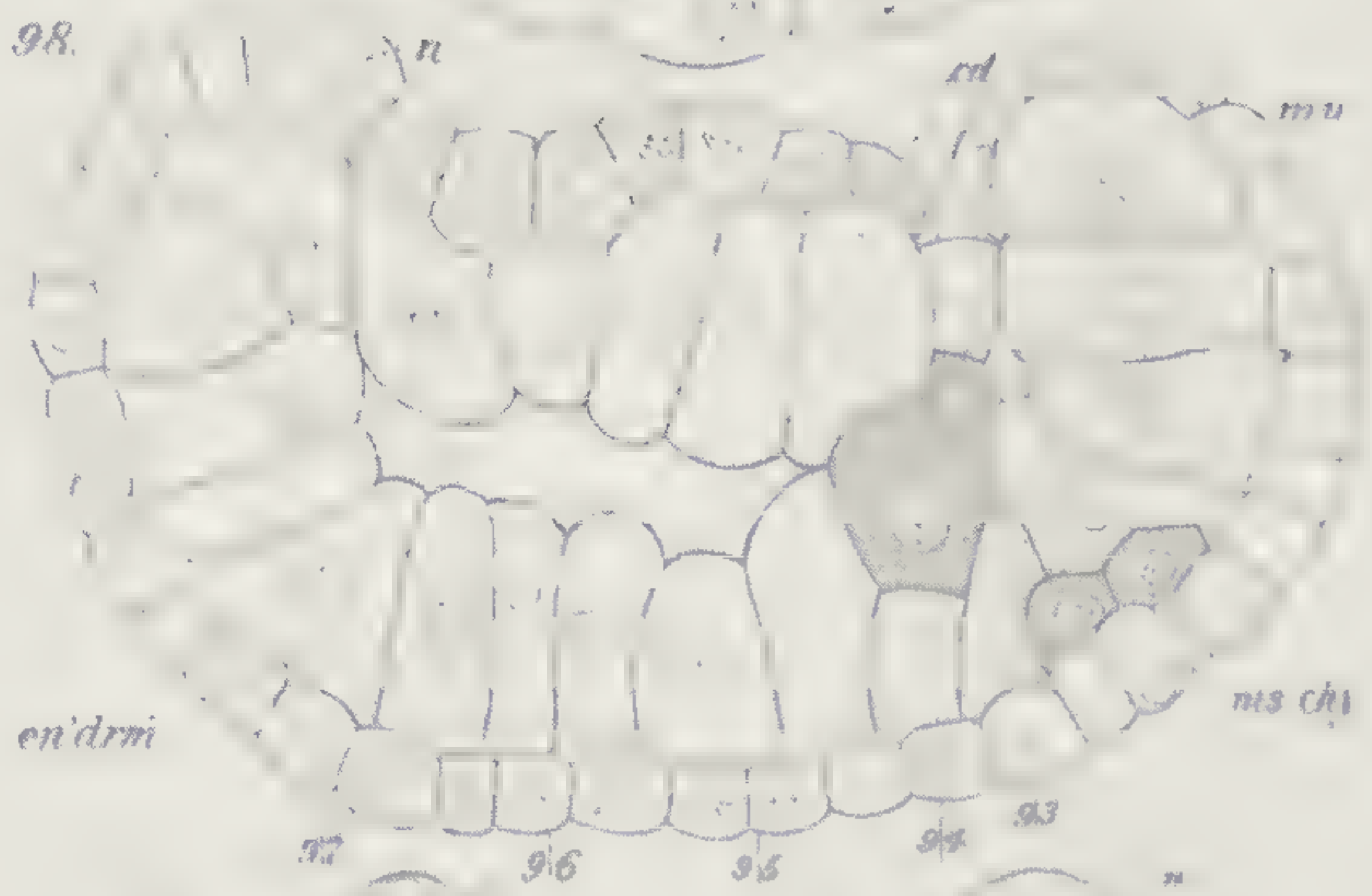
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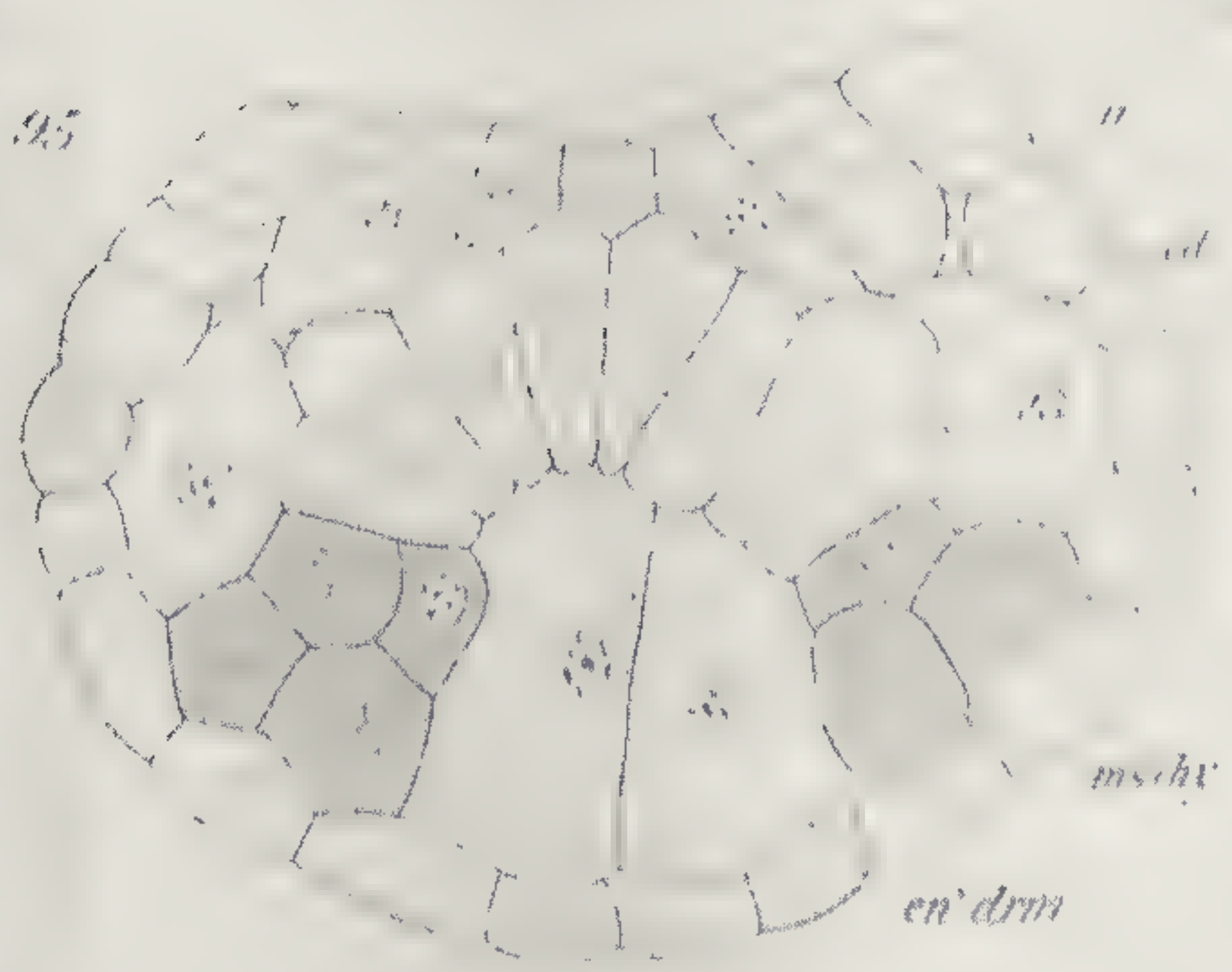
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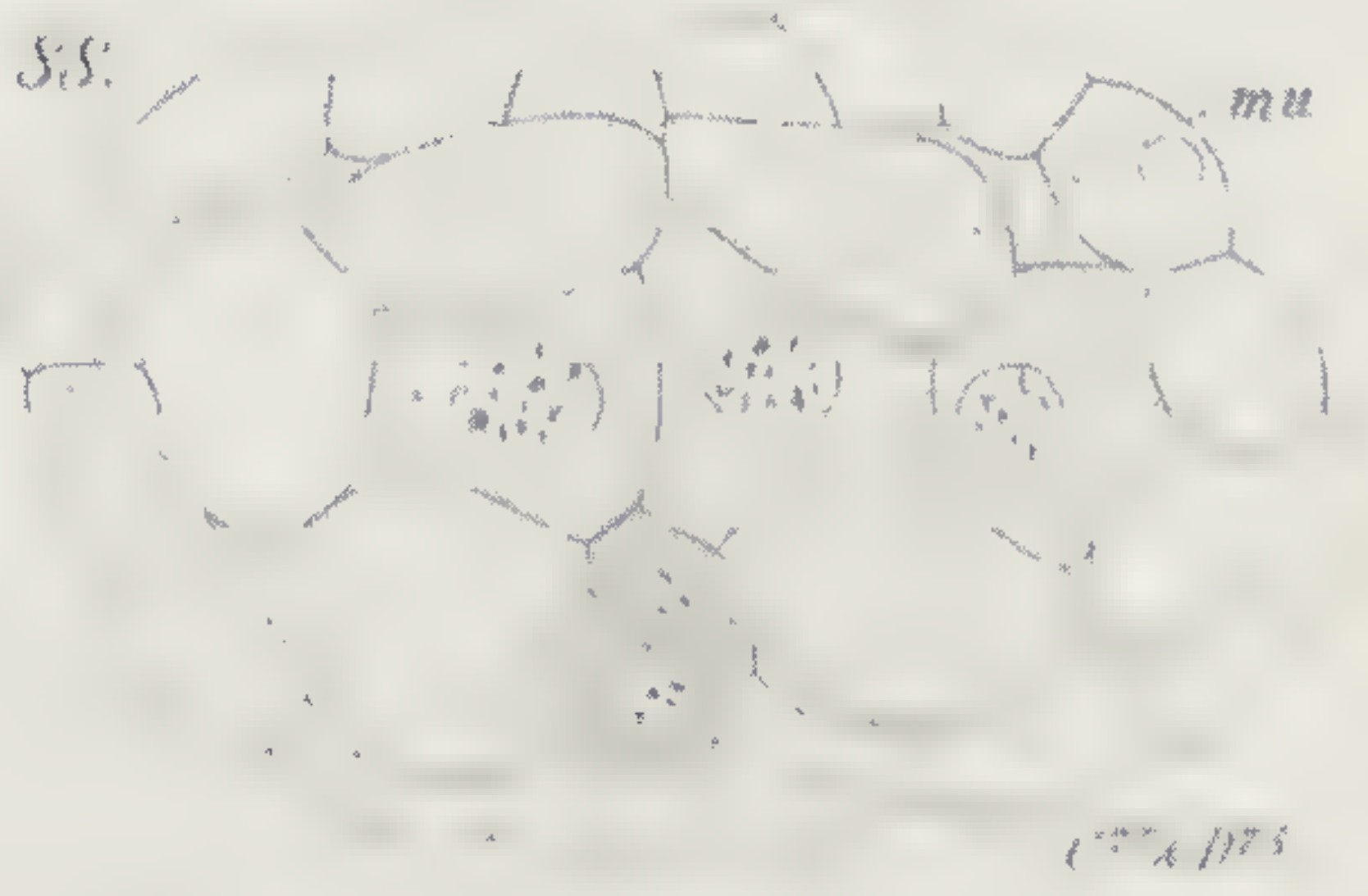

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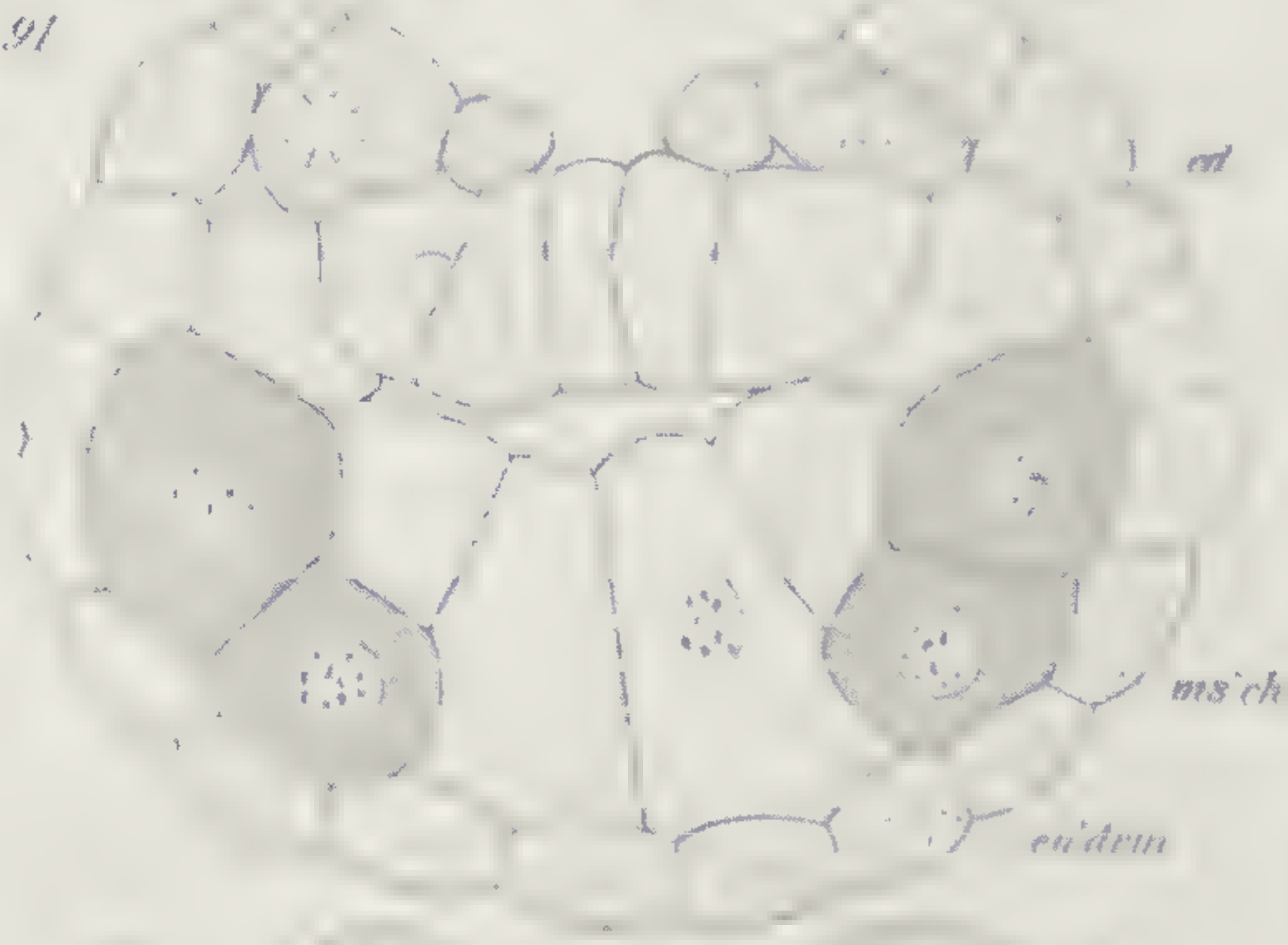
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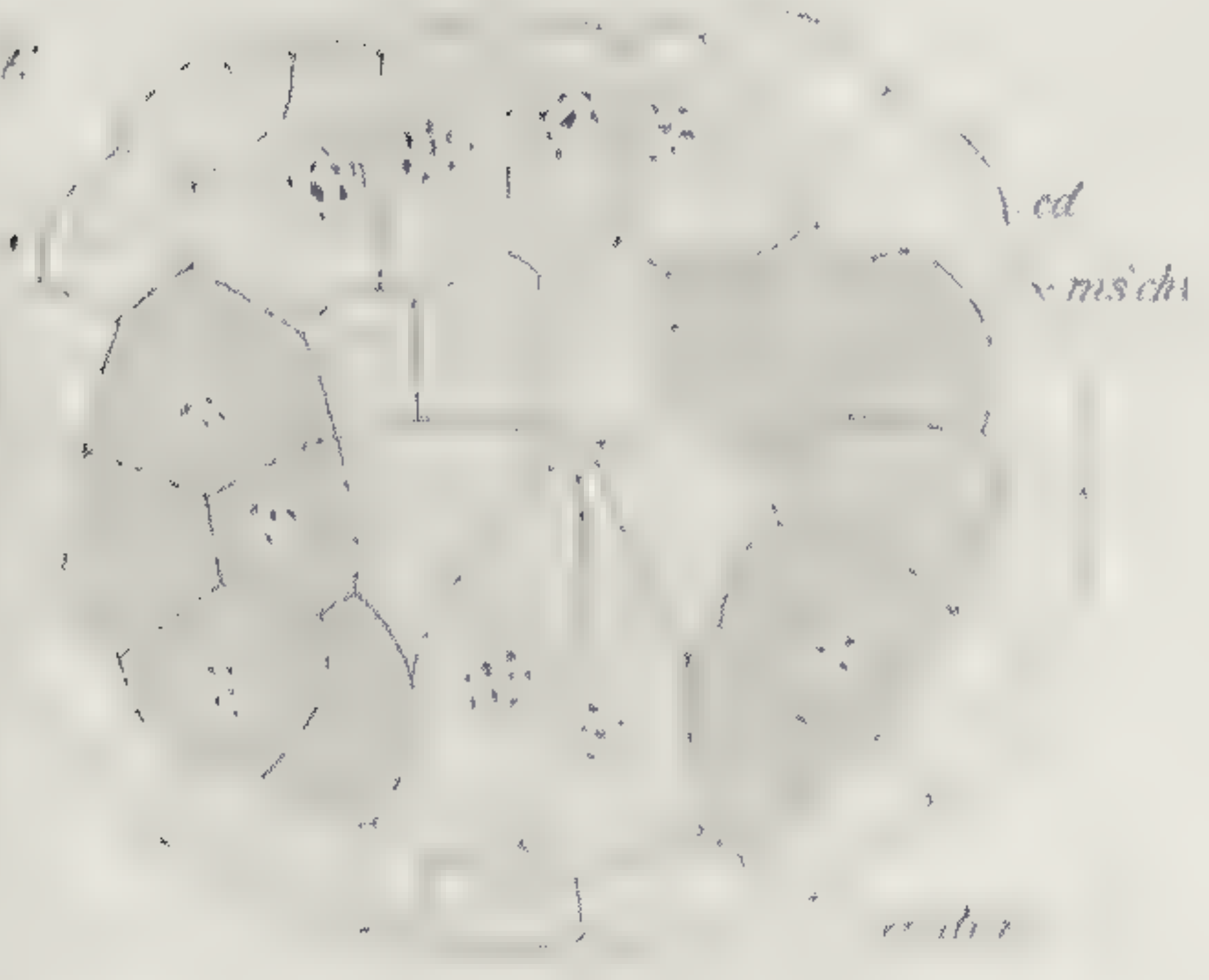

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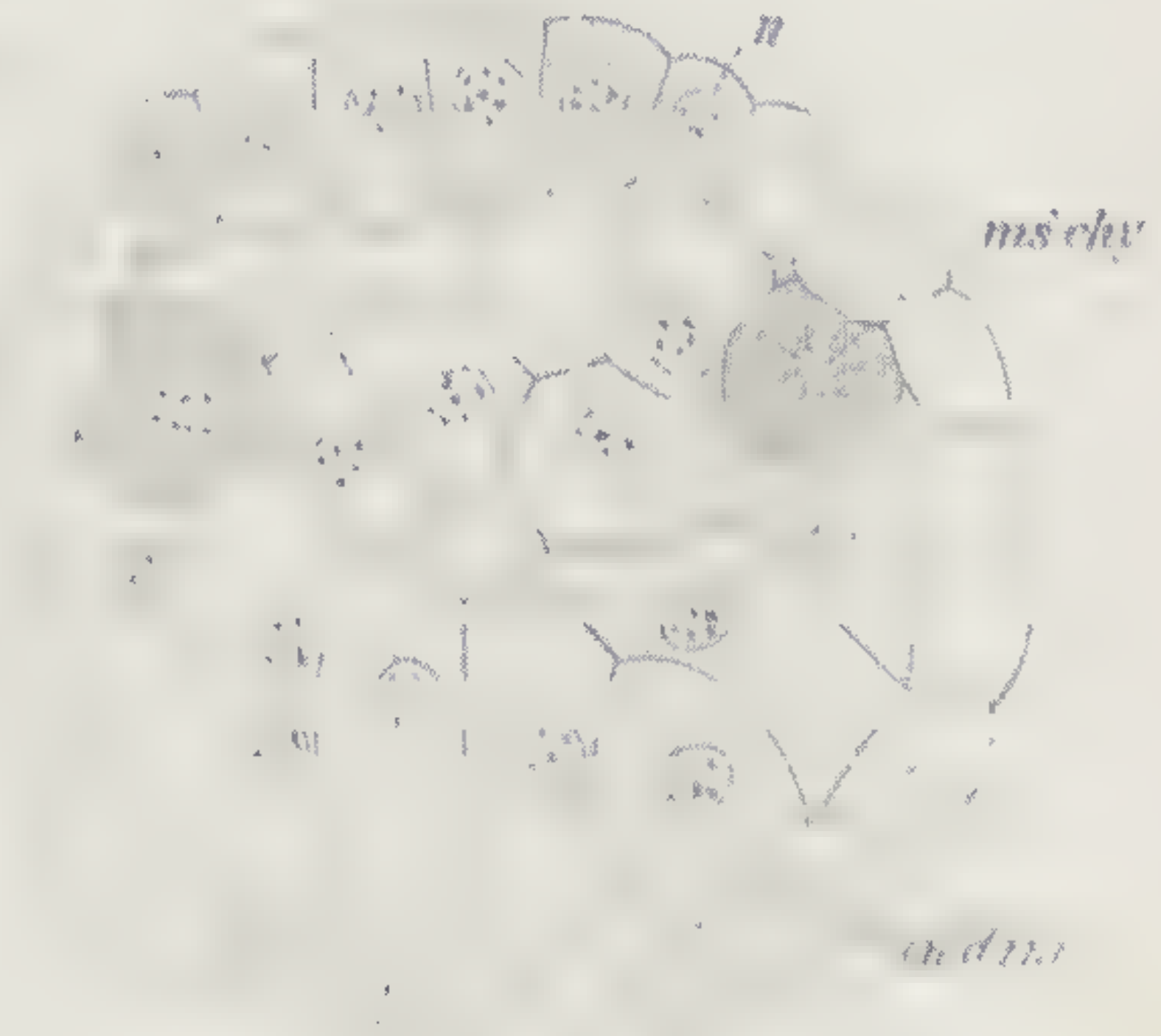

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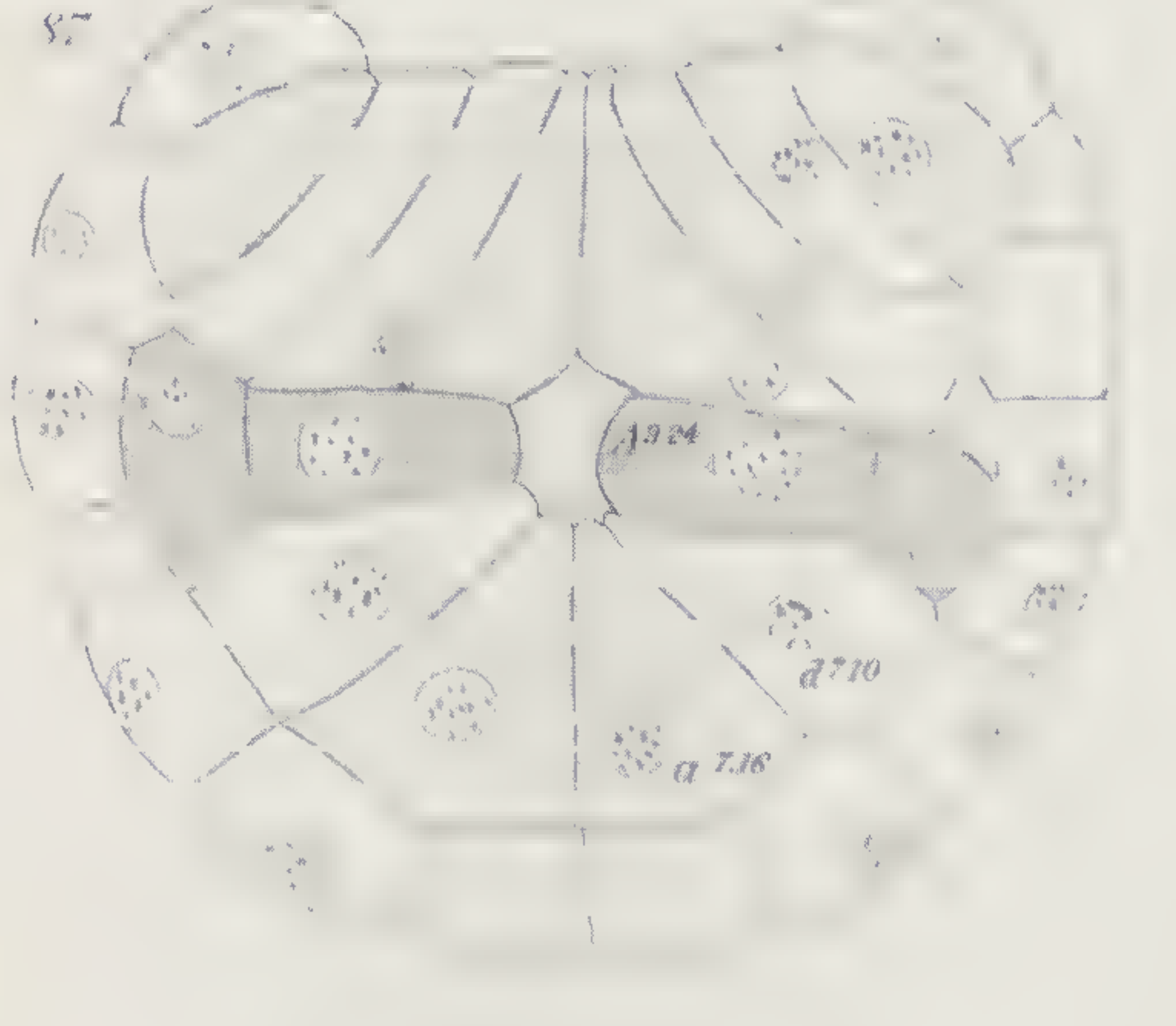
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a 7.16

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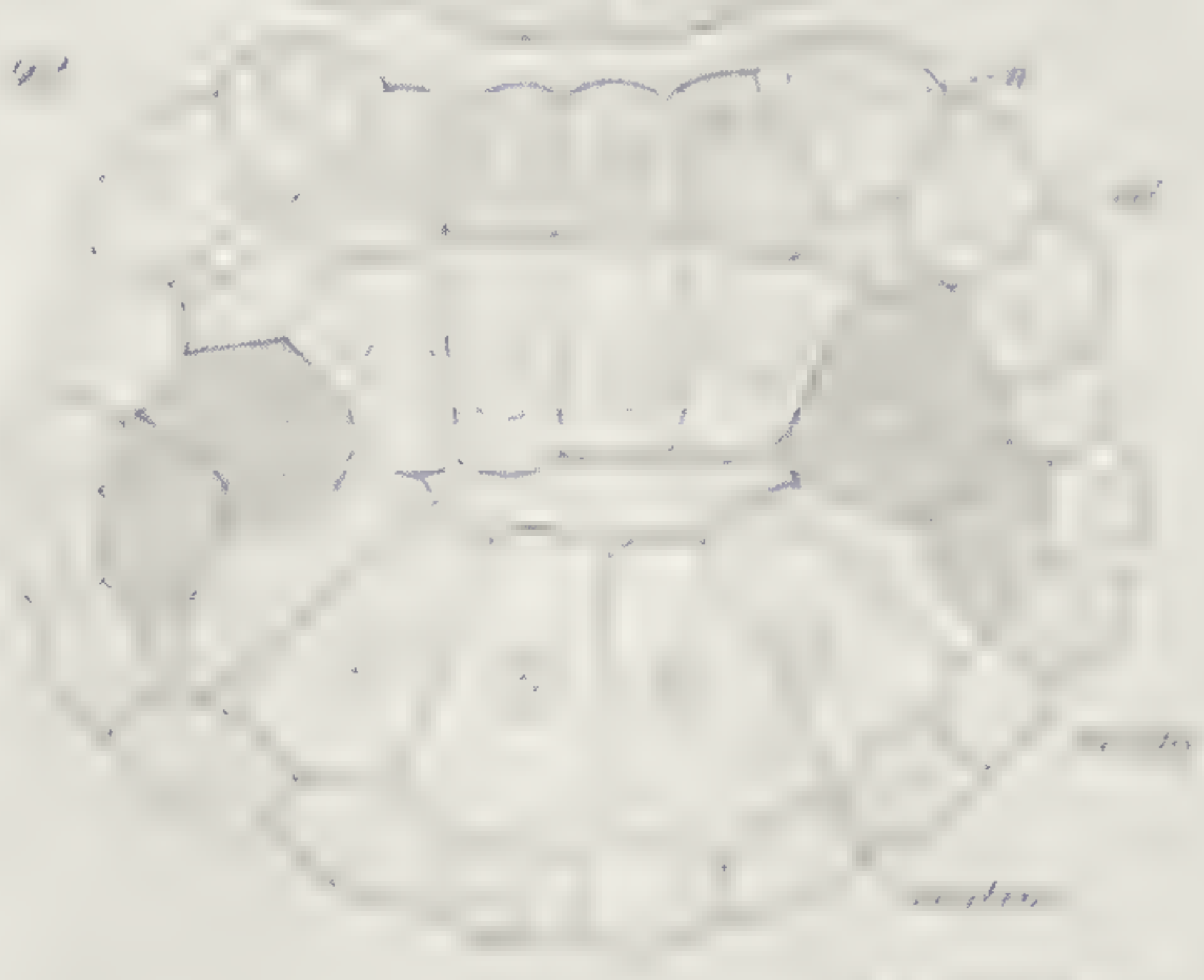


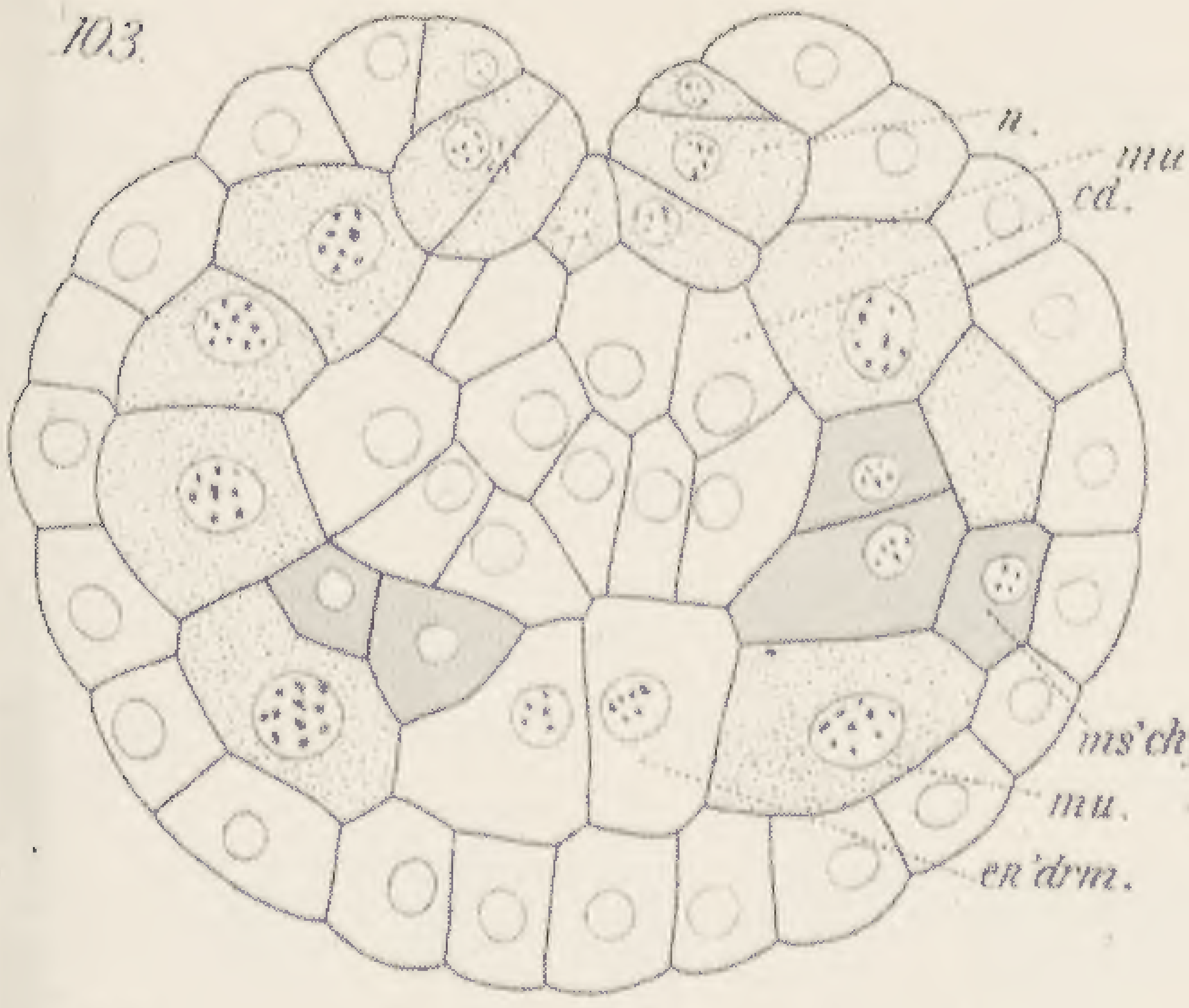
PLATE XIII.

Magnification of all Figures, 560 diameters.

Figs. 99–105. Seven sections from a series through an embryo with completely closed blastopore. Figs. 99–101, posterior to region of final closure of blastopore; Figs. 103–105, anterior to region of closure of blastopore.

Fig. 106. Cross section through an early larval stage (unhatched). The trunk region is shown in the left portion of the Figure, the tail region in the right portion.

103.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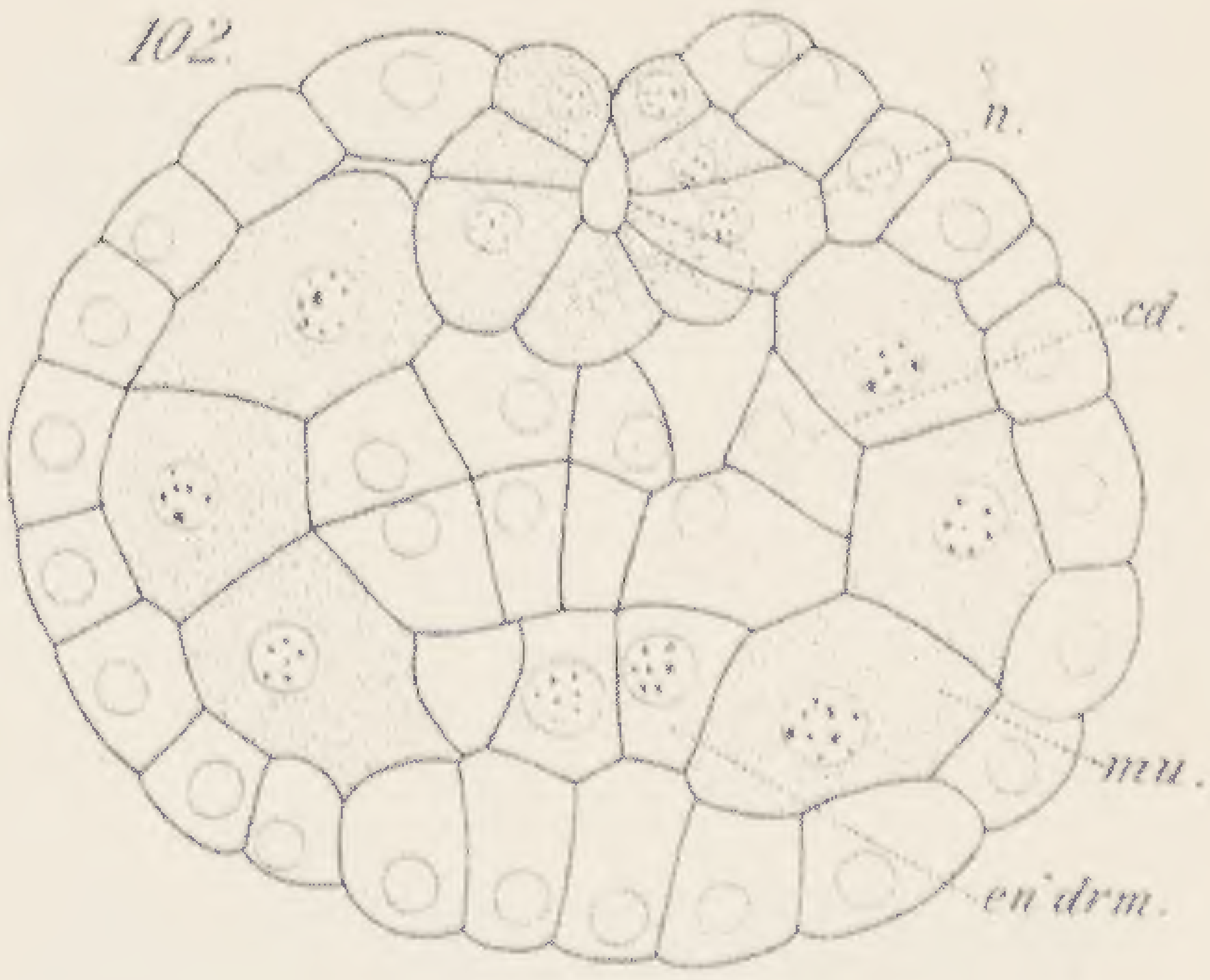
102.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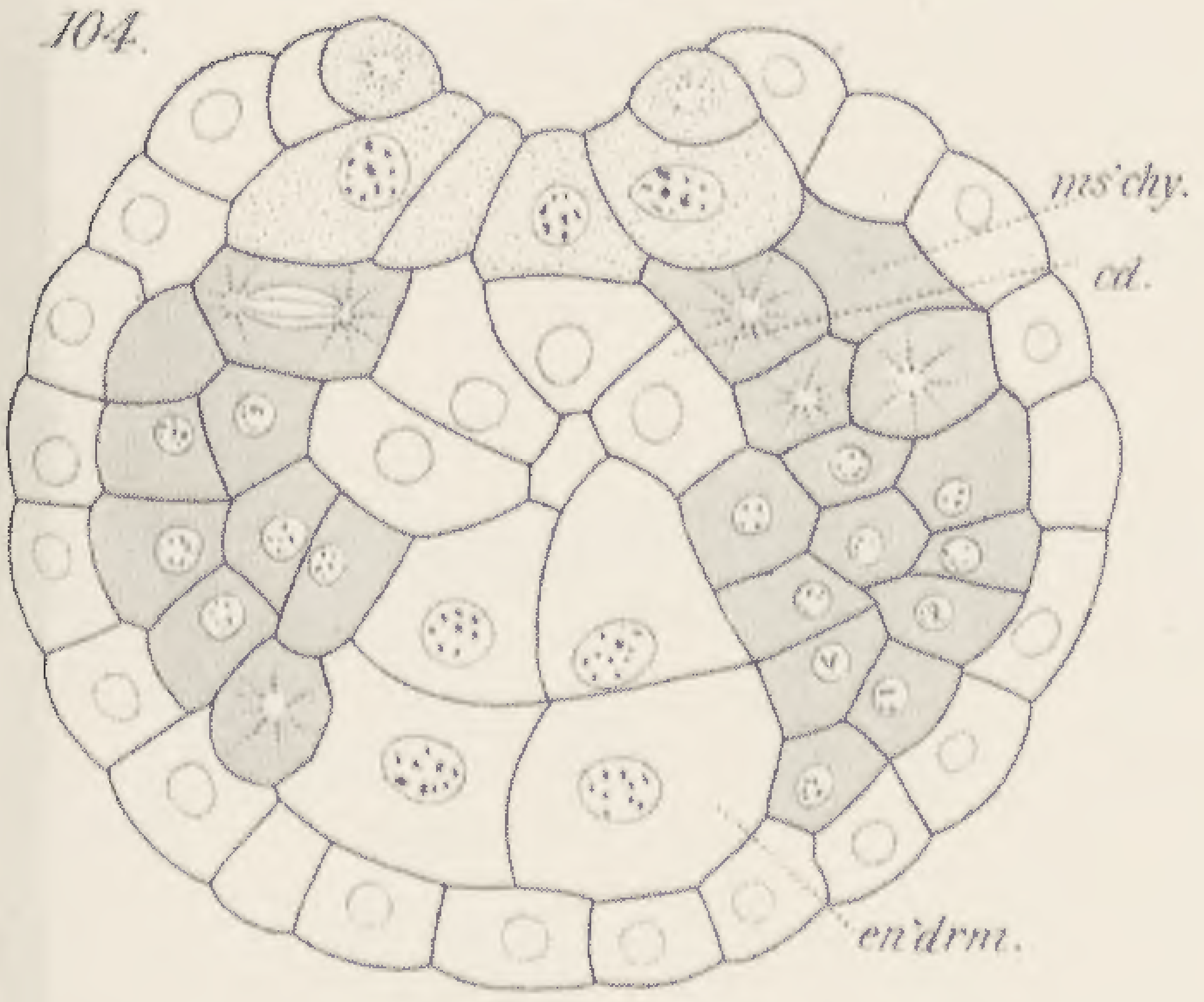
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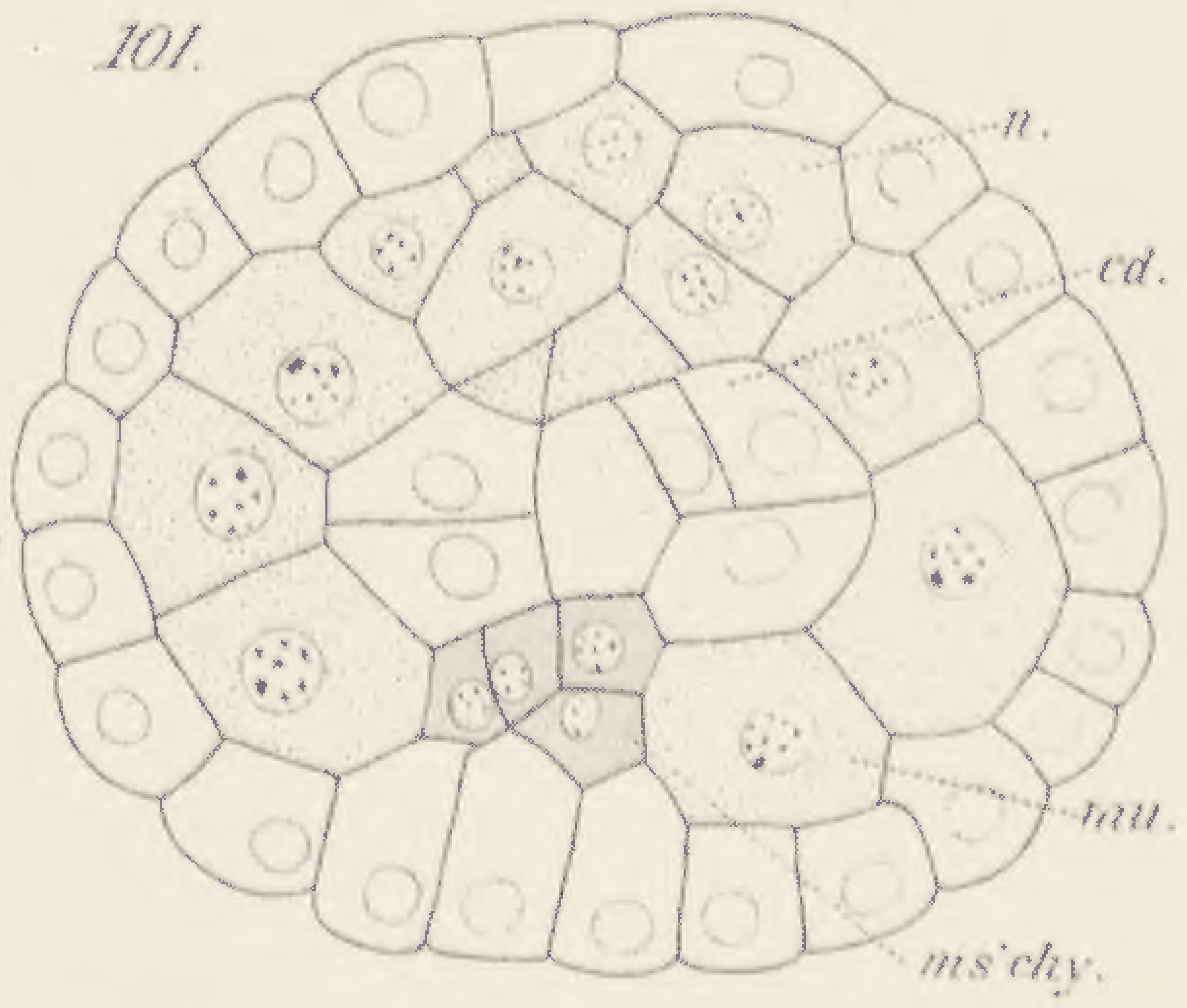
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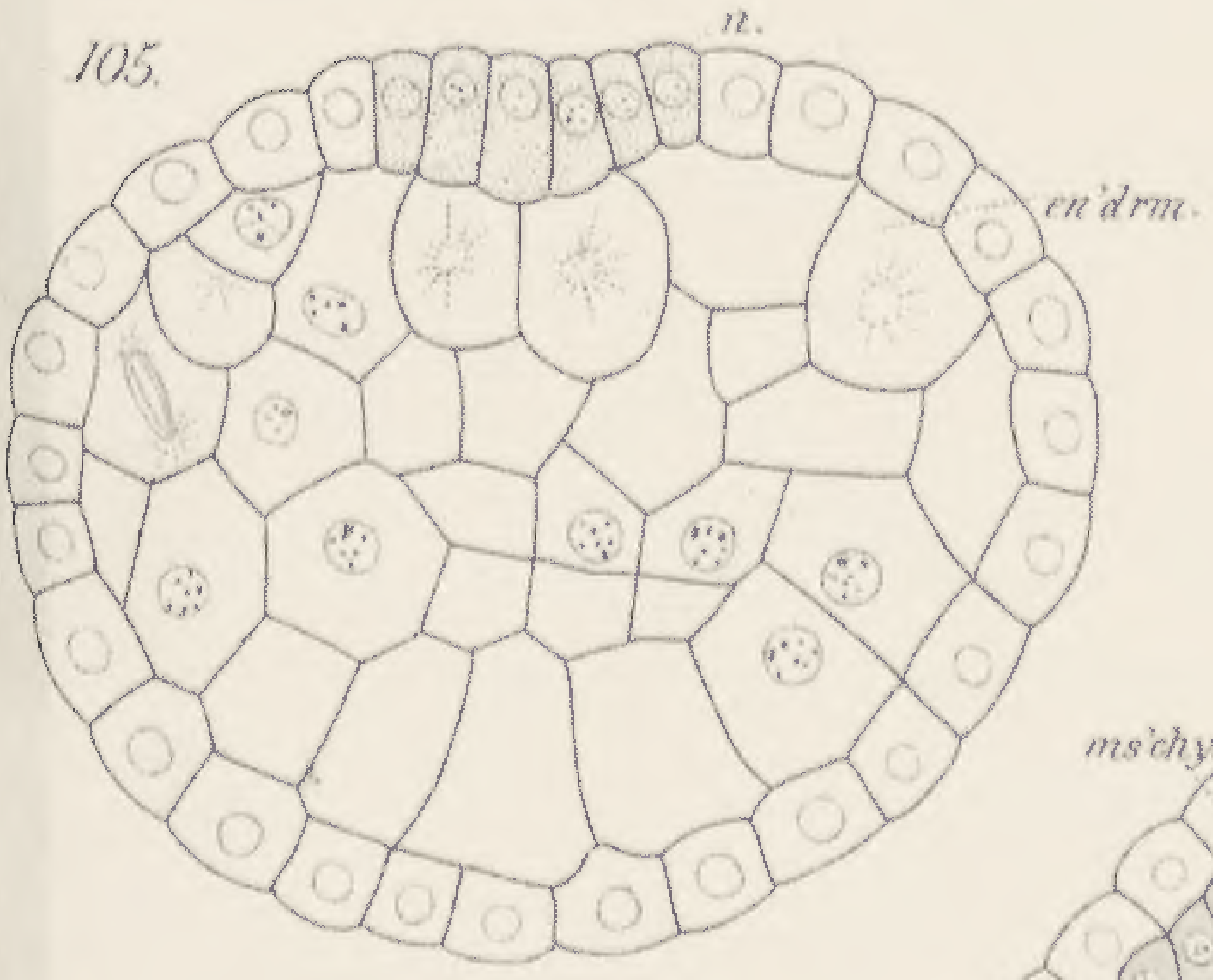

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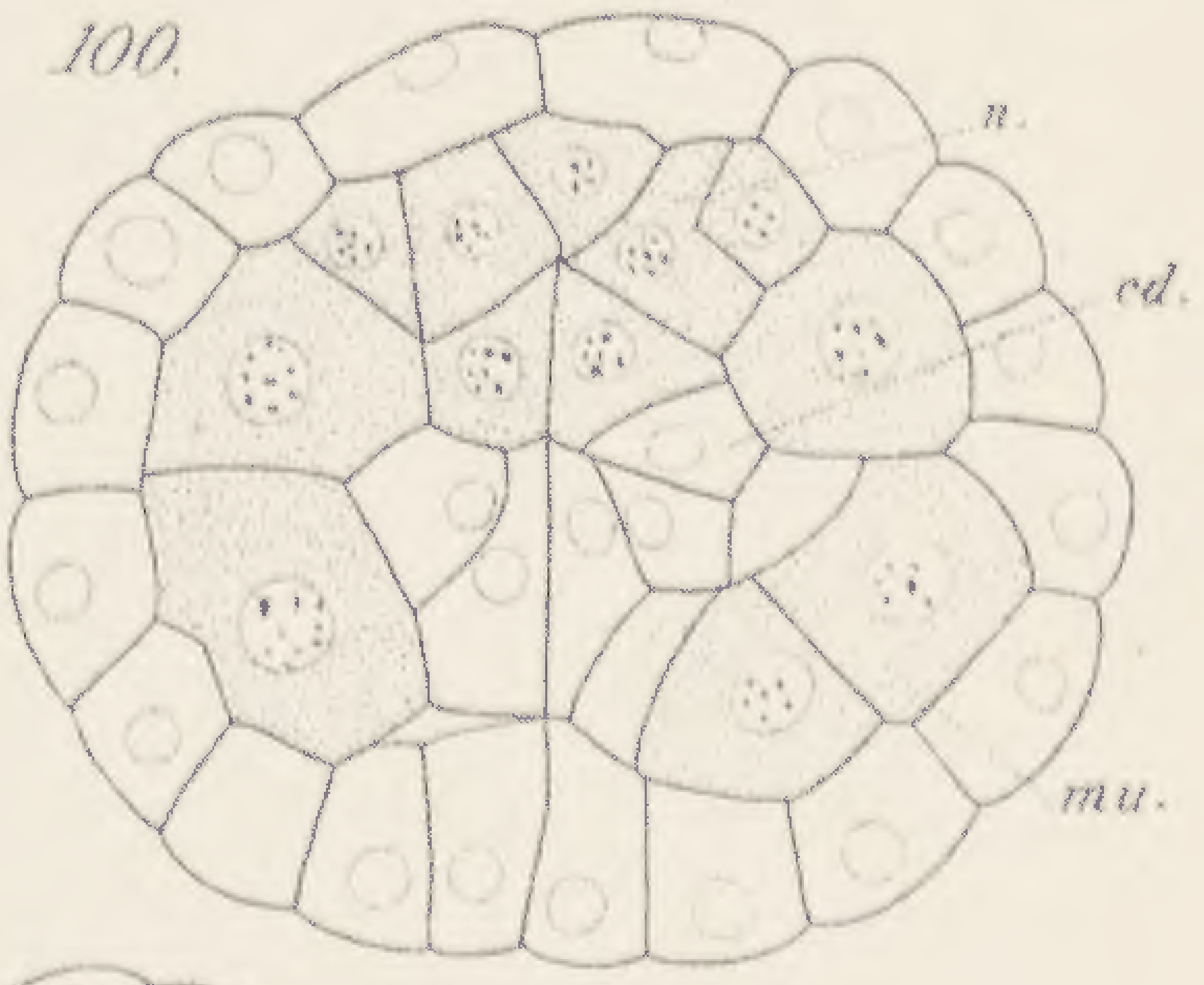

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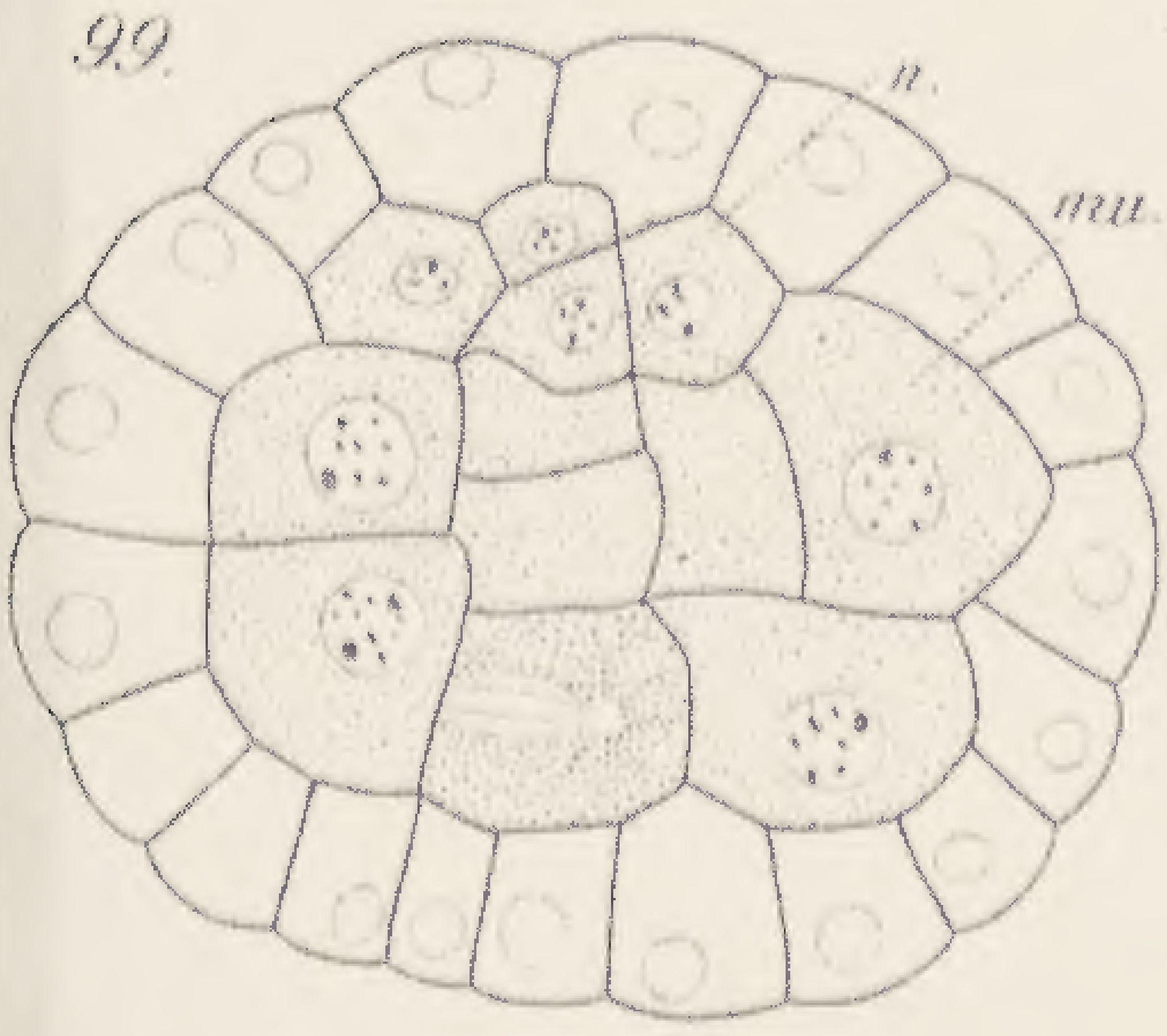

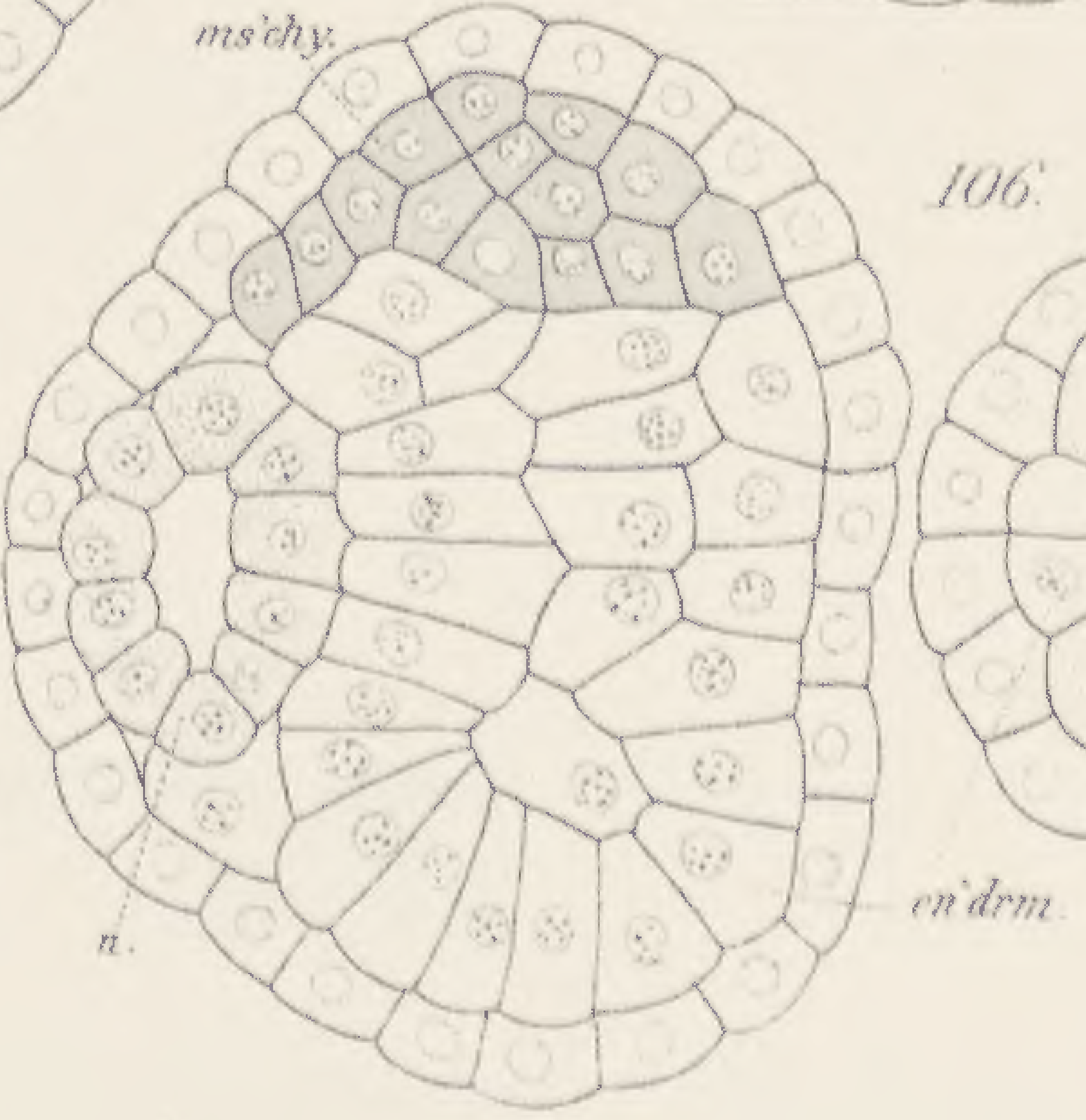
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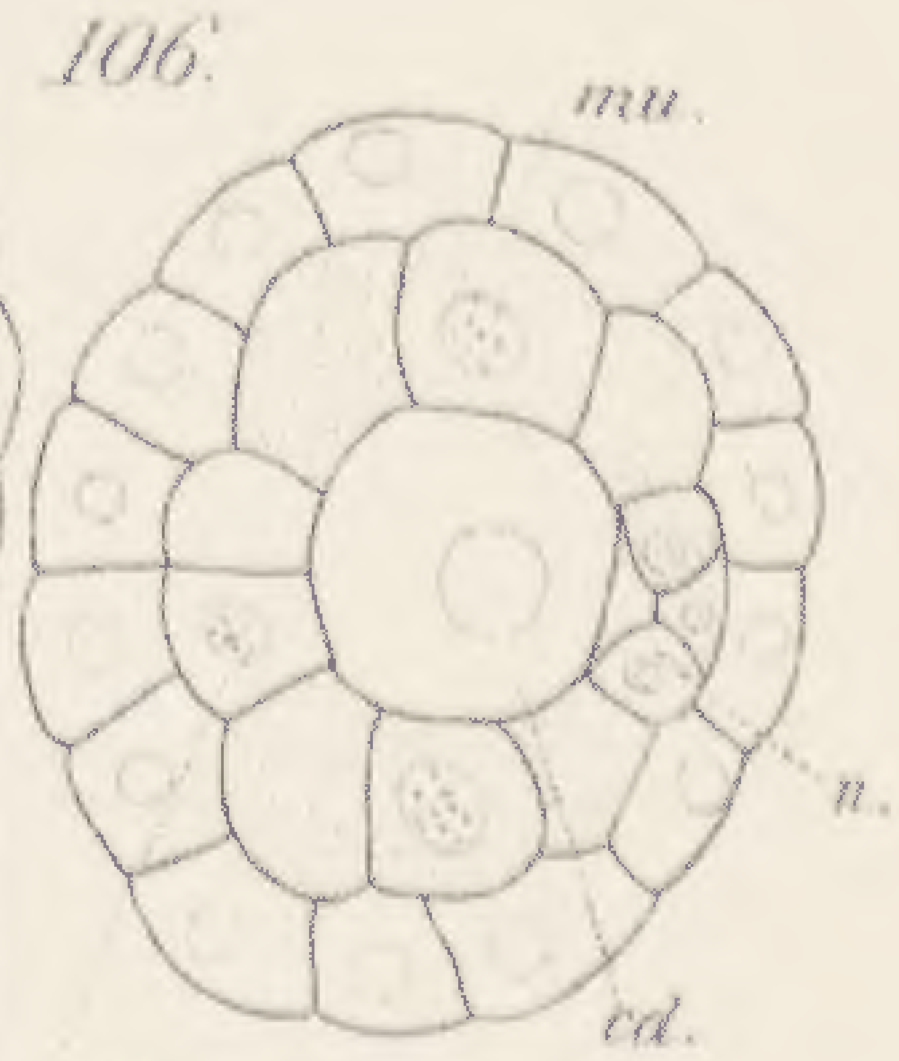
106.



BULLETIN

OF THE

MUSEUM OF COMPARATIVE ZOÖLOGY

AT

HARVARD COLLEGE, IN CAMBRIDGE

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